



UNIVERSITÀ DEGLI STUDI DI MILANO

DIPARTIMENTO DI CHIMICA

Ph.D. course in Chemistry — XXXIII cycle

Surface tailoring of oxide-based nanosystems for the design of advanced composite materials and smart devices

Ph.D. candidate:

Tommaso Taroni

Tutor: Dr Daniela Meroni

Coordinator: Prof. Emanuela Licandro

2020

Table of contents

Acknowledgements	5
Abstract.....	7
References	12
Oxides and their role in materials science	15
PART I Halloysite nanotubes.....	18
INTRODUCTION.....	18
I.1 General properties	18
I.2 Applications.....	21
CHAPTER 1 Halloysite nanotubes functionalization with phosphonic acids: role of surface charge on molecule localization and adsorption reversibility.....	32
1.1 Experimental procedures.....	34
1.2 Results and discussion.....	37
1.3 Conclusions	52
1.4 References	53
CHAPTER 2 Thiahelicene-grafted halloysite nanotubes: characterization, biological studies and pH triggered release	57
2.1 Experimental procedures.....	59
2.2 Results and discussion.....	63
2.3 Conclusions	72
2.4 References	73
CHAPTER 3 Composites of PA6 and halloysite nanotubes with improved hydrothermal ageing resistance: role of filler physicochemical properties, functionalization and dispersion technique	77
3.1 Experimental procedures.....	80
3.2 Results and discussion.....	83
3.3 Conclusions	100
3.4 References	101
PART II TiO ₂ porous films.....	108
II.1 General properties	108
II.2 Applications.....	115
CHAPTER 4 Bouncing droplets: a hands-on activity to demonstrate the properties and applications of superhydrophobic surface coatings.....	123
4.1 Experimental procedures.....	126
4.2 Results and discussion.....	127
4.3 Conclusions	132

4.4	References	132
CHAPTER 5 Wetting modification by photocatalysis: a hands-on activity to demonstrate photoactivated reactions at semiconductor surfaces		
		137
5.1	Experimental procedures	139
5.2	Results and discussion.....	140
5.3	Conclusions	143
5.4	References	143
CHAPTER 6 Synthesis of porous TiO ₂ films as photo-regenerating anti-fouling coatings for electrochemical sensors		
		146
6.1	Experimental procedures	147
6.2	Results and discussion.....	151
6.3	Conclusions	160
6.4	References	161
CHAPTER 7 Functionalization of porous TiO ₂ films for the development of nanostructured antimicrobial coatings.....		
		164
7.1	Experimental procedures	165
7.2	Results and discussion.....	168
7.3	Conclusions	176
7.4	References	176
PART III Superparamagnetic iron oxide nanoparticles.....		
		179
INTRODUCTION.....		
		179
III.1 GENERAL PROPERTIES.....		
		179
CHAPTER 8 Simple co-precipitation synthesis of USPIO suitable as T1 MRI contrast agents		
		191
8.1	Experimental procedures	193
8.2	Results and discussion.....	196
8.3	Conclusions	211
8.4	References	212
Conclusions and outlooks.....		
		216
Publications		
		218

Acknowledgements

I would like to thank the University of Milan for granting me this Ph.D. fellowship and providing the means to carry out my research. I am also grateful for having been selected and funded as a representant for the University at the LERU summer school organized in Edinburgh, July 2019.

In particular, I would like to thank my supervisor, Dr Daniela Meroni, and my co-supervisor, Prof. Silvia Ardizzone, for their continuous support and guidance throughout the course of my Ph.D.

My gratitude goes to the Delft University of Technology for hosting me and especially to Dr Laura Rossi, for supervising my work on superparamagnetic nanoparticles, discussed in Chapter 8.

I would like to acknowledge the contributions of Prof. Emanuela Licandro and Dr Silvia Cauteruccio (Università degli Studi di Milano), who provided the principal organic compounds presented in Chapter 2, namely 7,8-dipropyl-2-formyltetrathia[7]helicene and benzo[1,2-b:4,3-b'] dithiophen-2-carbaldehyde, beside participating to the writing of the relative paper. I would also like to express my appreciation to Dr Riccardo Vago, from IRCCS San Raffaele Scientific Institute, for his work on the in vitro tests presented in the same Chapter.

My most sincere gratitude also goes to Prof. Daniela Maggioni at Università degli Studi di Milano, who contributed to several surface characterizations reported in different chapters, as well as providing precious insights and suggestions on the preparation of iron oxide nanoparticles, presented in Chapter 8.

Prof. Luigi Falciola and Dr Valentina Pifferi at Università degli Studi di Milano deserve recognition for their crucial contribution to the electrochemical characterization of TiO₂ porous films, presented in Chapters 6 and 7.

I would like to extend my gratitude also to Dr Valentina Sabatini, then at Università degli Studi di Milano, who performed all polymer syntheses and some of the characterizations reported in Chapter 3, other than being the main contributor to writing the relative paper. Moreover, she also oversaw the synthesis of polystyrene nanospheres presented in Chapters 6 and 7.

Many thanks go to Dr Mariangela Longhi at Università degli Studi di Milano, who optimized the synthesis of the SiO₂ nanoparticles presented in Chapter 1.

I would like to thank Elettra Sincrotrone Trieste for granting me and my colleagues 24 shifts at the SAXS beamline. Many thanks also to Dr Stefano Franchi, for his contribution to the NEXAFS surface characterizations presented in Chapter 2.

Lastly, I would like to express my gratitude to my Ph.D. colleagues, Luca Rimoldi and Carolina Cionti, for their significant contributions to Chapters 5 and 7 respectively.

Abstract

My Ph.D. project focused on the tailoring of the surface properties of oxide substrates for the preparation of advanced composite materials and devices. The initial focus of my research activity was the surface modification of halloysite nanotubes (HNT), a natural material with unique structural features. I then extended my investigation to other oxides (titanium dioxide, TiO_2 , and Superparamagnetic Iron Oxide Nanoparticles, SPION), applying the surface modification approaches developed for HNT substrates. The resulting oxide-based hybrid systems showed promising properties as stimuli-responsive devices for health and environmental applications, and as fillers for polymeric nanocomposites with enhanced durability. The main results obtained for each oxide material will be presented in the following sections.

1. Halloysite nanotubes (HNT)

Halloysite is a polymorph of kaolinite which naturally wraps itself to form tubular structures (Figure 1A). It is one of the few nanotubular systems presenting an inner lumen and an outer surface characterized by different surface charge and structural composition: the internal surface exposes aluminium hydroxyl groups, while the outermost layer is silica[1]. Among its many potential applications, its tubular dual structure has sparked interest in the field of nanomedicine and polymer nanocomposites[2]. However, only few reports investigated the possibility of a selective functionalization of the inner and outer HNT surfaces. During my first year of PhD, I investigated the selective functionalization of HNT with phosphonates, hetero-organic compounds bearing a C-PO(OH)_2 group, as potential site-specific linkers for the grafting of active molecules. I took advantage of the phosphonic acid selectivity towards certain oxides (especially aluminium oxides) to achieve surface-specific functionalization of HNT. An in-depth comprehension of such hybrid systems is no trivial matter, as the inner and outer HNT surfaces possess different accessibility and reactivity but are not separable. For this reason, beside HNT, I used purposely prepared model oxides, mimicking the inner and outer nanotube surfaces, to better study the actual selectivity towards SiO_2 and AlOOH exhibited by the phosphonate moiety. Octylphosphonic acid (OPA) was chosen as functionalizing agent, as its alkyl chain allowed me to monitor the surface modification through changes in the water contact angle (θ). I found that the oxide isoelectric point (pH_{IEP}) plays a major role in determining a stable OPA adsorption: while AlOOH showed good reactivity towards OPA, SiO_2 , which is negatively charged at the impregnation pH (pH 4), did not react with the phosphonate heads. The functionalization reversibility was also assessed: Samples showed OPA release at pH values more alkaline than the oxide isoelectric point, when the surface charge is negative. Overall, these results support an OPA-oxide bond governed both by electrostatic and covalent forces. The selective functionalization of HNT inner lumen was also demonstrated via a combination of characterization techniques, including FTIR spectroscopy, ζ -potential measurement and water dispersibility assay.

Another scantily investigated topic regarding HNT nanosystems is their covalent modification. Covalent grafting allows for a superior control over the release kinetics of active principles, as the initial release burst observed in the case of electrostatically bound compounds is greatly reduced. In this respect, I studied the covalent attachment of a biologically active molecule to HNT via an imine bond. This type of covalent bond was chosen to open up the possibility for a controlled release of the bioactive molecule activated by pH changes. To this purpose, HNT was functionalized with (3-aminopropyl)triethoxysilane (APTES), a bifunctional linker which can cover the oxide surface with amino groups. Despite being non-selective for either of the two phases, it was chosen based on the relative ease with which it can be grafted to the substrate. Then, tetrathia[7]helicene aldehyde (7-THA) was bound to HNT via APTES, exploiting imine chemistry. 7-THA belongs to a group of polyaromatic molecules capable, thanks to their peculiar helicoidal shape, of intercalating DNA strands, which is at the basis of anti-sense therapy[3]. Nonetheless, HNT abysmal water solubility highly reduces their bioavailability and makes the use of hydrophilic nanocarriers necessary. I tested the relative release efficiency of 7-THA under slightly different pH conditions, representative of lysosomal, tumoral and healthy extracellular pH values. The resulting HNT-7TH system was studied in detail via XPS and angle-resolved near edge X-ray absorption fine structure spectroscopy (NEXAFS) at the Material Science Beamline of the Elettra synchrotron facility. The latter characterization technique required the synthesis and functionalization of oxide films replicating HNT surfaces, in order to probe the orientation of a population of adsorbed molecules with respect to the surface plane. NEXAFS results suggested a preferential orientation of the aromatic rings of helicene normal to the oxide surface, possibly as a result of π - π stacking interactions (Figure 1B). XPS analyses were performed at each stage of the preparation process of HNT-7TH and after the release test at pH 5 (Figure 1C): the observed change in the elemental composition indicated a release of 70% of the loaded 7-THA as a result of pH change. Given the interest for tumour therapy applications, *in vitro* tests were carried out to assess the selectivity of this system on cancer cells. The effect of HNT-7TH on the viability of bladder cancer cells was tested by Dr Riccardo Vago at the IRCCS San Raffaele Scientific Institute. Two different cell lines, named 5637 and HT-1376, with an extracellular pH of 7.2 and 6.8 respectively, were subjected to increasing concentrations of HNT-7TH and bare HNT as control. HNT-7TH was found to cause a more marked reduction in cell viability on the HT-1376 cell line, suggesting a faster release of the cytotoxic 7-THA at slightly acidic pH values. Release kinetics also supported this hypothesis: a new model system utilizing benzodithiophene (BDT), a more water-soluble mimic of 7-THA, was prepared analogously to the HNT-7TH powders. The release of BDT was monitored via UV-vis spectroscopy at pH 5.0, 6.8 and 7.4. The amount of released BDT at pH 7.4 was negligible even after 48 h; decreasing the pH value to 6.8 visibly increased release rates, while the release efficiency was highest for the treatment at pH 5.0.

The natural origin of HNT causes a marked variability in its physicochemical features, such as its morphology and surface charge, depending on the extraction site[4]. I investigated this aspect, often overlooked in the literature, with respect to the integration of HNT as nanofillers in polymer composites. Halloysite has been investigated as filler owing to its low cost, thermal and mechanical resistance, and high aspect ratio, which is crucial to guarantee strong polymer-filler interactions[5]. In this regard, I investigated

also the effect of surface functionalization in promoting HNT compatibility with the chosen polymer matrix, Polyamide 6 (PA6). PA6 has a broad range of applications, from the automotive sector to the textile industry, owing to its good mechanical performances and high thermal resistance. However, when exposed to humid conditions, it suffers from degradation in a matter of few weeks[6], as water can interpenetrate within the hydrogen bonds between -NH and C=O groups and disrupt them, leading to the loss of tensile strength and elasticity[6–9]. The addition of nanotubular fillers represents a viable strategy for overcoming this issue, although the additive/polymer interface at high filler content can become a privileged site for moisture accumulation[10]. For this reason, HNT were added to PA6 in very low amounts ($< 5\%$ w). The roles played in the reinforcement of the polymer by the physicochemical properties of HNT from two different sources and their functionalization with APTES were investigated in composites prepared by two different dispersion techniques (*in situ* polymerization *vs.* melt blending). The aspect ratio (5 *vs.* 15) and surface charge (-31 *vs.* -59 mV) of the two HNT samples proved crucial in determining their distribution within the polymer matrix: both *in situ* and melt blending dispersion techniques showed that lower surface area, higher aspect ratio and greater surface charge enhance filler incorporation and improve the final composite performance. Finally, filler surface modification with APTES played a major role in the durability of the PA6-HNT nanocomposites: after 1680 h of hydrothermal ageing, functionalized HNT reduced the diffusion of water into the polymer, lowering water uptake after 600 h up to 90%, increasing the materials durability. Positive effects could also be measured regarding the molecular weight distribution and rheological behaviour. These improvements could be related to the presence of amino groups on the HNT surface, which lowered the filler surface energy and prevented the diffusion of water molecules into the nanocomposites[11].

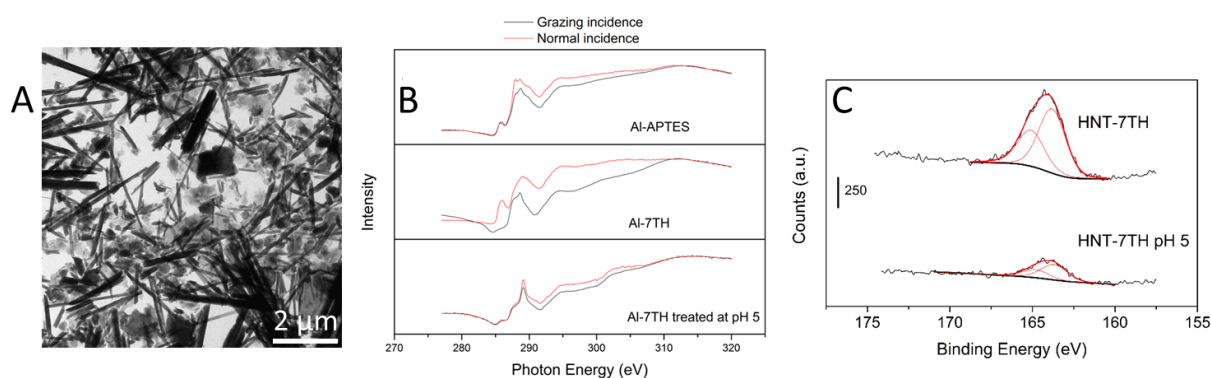


Figure 1 – Halloysite morphology captured by TEM imaging (A); the release of 7-THA was followed on model oxides via synchrotron light NEXAFS spectroscopy (B) and on HNT through XPS, monitoring the S 2p region (C).

2. Titanium dioxide

TiO_2 is arguably the most extensively investigated semiconductor for photocatalytic applications, from solar cells to pollutant abatement. However, applications of TiO_2 for the preparation of smart surfaces are comparatively less common. My research interest in TiO_2 started with the preparation of surfaces with controlled wetting features. First,

the surface of commercial TiO_2 powders was functionalized with perfluorinated alkylsilanes. Then, I employed the photocatalytic features of TiO_2 films to produce patterned surfaces with superhydrophilic/superhydrophobic contrast by means of photocatalytic lithography.

During the course of my second year of PhD, I focused on the deposition of TiO_2 films with controlled porosity followed by their surface modification to impart them functional properties. These systems were applied as photo-renewable coatings for electrochemical sensors and stimuli-responsive surfaces for the controlled release of active compounds. Control over film porosity was achieved by a hard-template approach using polystyrene (PS) nanospheres of different sizes, both commercial and synthesized in-house following a classical procedure[12]. Different film deposition strategies were investigated.

First, I deposited a TiO_2 layer on top of a porous SiO_2 coating on a FTO electrode. Extensive characterization via cyclic voltammetry showed that the addition of a TiO_2 layer increased peak currents due to the promoted diffusion of the analyte in the porous structure, driven by capillary effects[13] (Figure 2A). Furthermore, the TiO_2 layer promoted the light-activated regeneration of the electrode surface after having been fouled. Starting from there, I investigated the possibility of pure TiO_2 mesoporous films, a task made difficult by the intrinsic incompatibility between an alcohol-based TiO_2 sol, extremely prone to hydrolysis, and a water-based PS suspension. This issue was solved by either adopting an aqueous TiO_2 sol, slowly evaporated in presence of the particle templates (Figure 2B), or by performing a solvent exchange procedure on the PS suspension. The latter procedure resulted in pure TiO_2 films with easily tuneable thickness and homogeneous porosity, opening the door to a fine tuning of the cyclovoltammetric response. The self-cleaning features of the pure TiO_2 films were also tested by purposely fouling their surface with long-alkyl chain substituents: a fast and complete regeneration of the surface was achieved upon irradiation with UV light.

The prepared pure TiO_2 porous films were also used as substrates for the loading of bioactive substances. Cinnamaldehyde, a natural substance known for its antimicrobial properties but unstable in environmental conditions, was anchored to the film surface via APTES linkers through an imine bond, using a protocol similar to the one developed for 7-THA-loaded HNT. The immobilized cinnamaldehyde proved stable to environmental conditions for months and tests of pH-triggered release performed at pH 5.5 and 7.4, showed a faster release at lower pH values. Finally, the photoactive nature of the oxide substrate could be used to promote the self-cleaning of the fouled surface after usage: after UV-light regeneration, the TiO_2 film could be functionalized anew and reused.

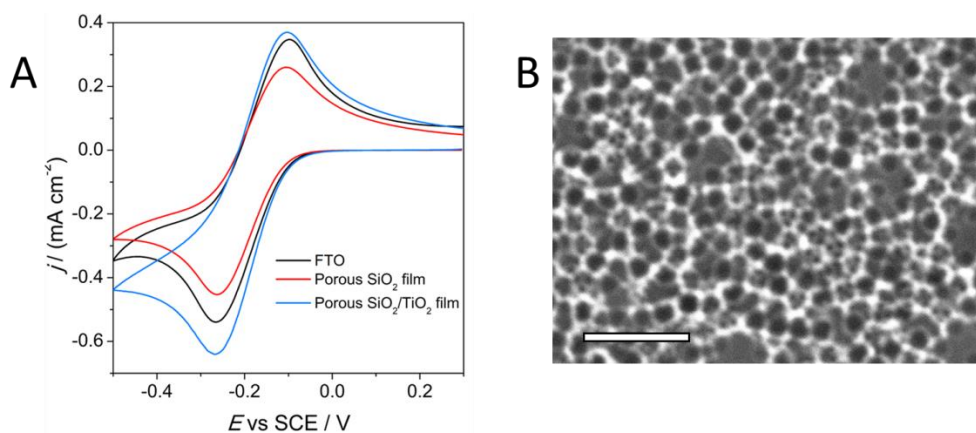


Figure 2 – Cyclic voltammograms of composite SiO₂/TiO₂ films (A); SEM image of a porous TiO₂ film (scale bar is 4 μm) (B).

3. Superparamagnetic Iron Oxide Nanoparticles (SPION)

During my third year of PhD, I spent six months at the Technische Universiteit Delft, Netherlands, in the Advanced Soft Matter group, under the supervision of Dr Laura Rossi. There my research focused on the synthesis and surface modification of Ultrasmall SPION.

SPION have gained increasing attention thanks to their peculiar behaviour: being smaller than a single magnetic crystal domain, they are free to rotate unless a specific orientation is induced by an external field. Due to their magnetic properties, they can be adopted in hyperthermia, drug delivery and as contrast agents (CA) for magnetic resonance imaging (MRI)[14]. Contrast agents are commonly used to speed up either T1 or T2 relaxation, enhancing the local contrast in pathological tissue to produce more detailed images. T1 CA are commonly represented by gadolinium complexes, while SPION are generally adopted as T2 CA. The first are preferred by radiologists, while the latter are less favoured because the darker tones they provide can be mistaken with low resolution and background interference[15,16]. Nonetheless, Gd-based CA present a serious health risk for those patients unable to efficiently remove these heavy metal complexes due to pre-existent kidney or liver pathologies[17]. While most SPION act as T2 CA, several papers report their potential use as T1 CA if their size is sufficiently small, indicatively less than 4 nm[18,19]. These materials are known as Ultrasmall SPION, or USPION.

My aim was to develop a synthetic protocol to prepare USPION suitable as T1 contrast agents via co-precipitation, to minimize synthetic requirements. To this purpose, the influence of several parameters such as reaction temperature, base type, purification procedure, stabilizing agent and precursor concentration was investigated. Particles were synthesised at room temperature (RT) and 50°C, using NH₄OH, N(CH₃)₄OH or NaOH as base. Particle purification was performed via magnetic decantation, centrifugation and dialysis against different solutions (water, citric acid and sodium citrate solutions). Sodium oleate, (3-aminopropyl)triethoxysilane (APTES) and citric acid were tested as stabilizing agents and precursor concentration was varied between 1 M and 0.5 M. It was found that the best results were obtained at room temperature and that the peptizing effect of the tetramethylammonium ion is crucial to guarantee an

optimal colloidal stability, making $\text{N}(\text{CH}_3)_4\text{OH}$ the base of choice. The concentration of starting precursor solutions proved to be the determining factor acting on particle size, as halving it led to a narrow particle size distribution centred around 3 nm, a significant shift from the starting 7 nm (Figure 3). Centrifugation was ineffective when adopted to wash the nanoparticles, but it proved a promising size-selection tool that could be combined with dialysis in an efficient work up protocol. Dialysis proved to be the most efficient technique to remove potentially toxic impurities, but it negatively impacted the colloidal stability, which could be mitigated by the use of a proper stabilizing agent. To preserve a high colloidal stability even after the removal of $\text{N}(\text{CH}_3)_4\text{OH}$, surface modification with several stabilizing agents was tested. Among the tested molecules, citric acid was the only one to show positive effects on particle size and aggregation, more so when added before the start of particle nucleation. These results represent a promising advance in the development of efficient T1 contrast agents based on USPIOs in terms of lowering the synthetic requirements: monodisperse magnetic nanoparticles were prepared through a simple co-precipitation procedure, performed at room temperature, without the aid of any polymeric additive.

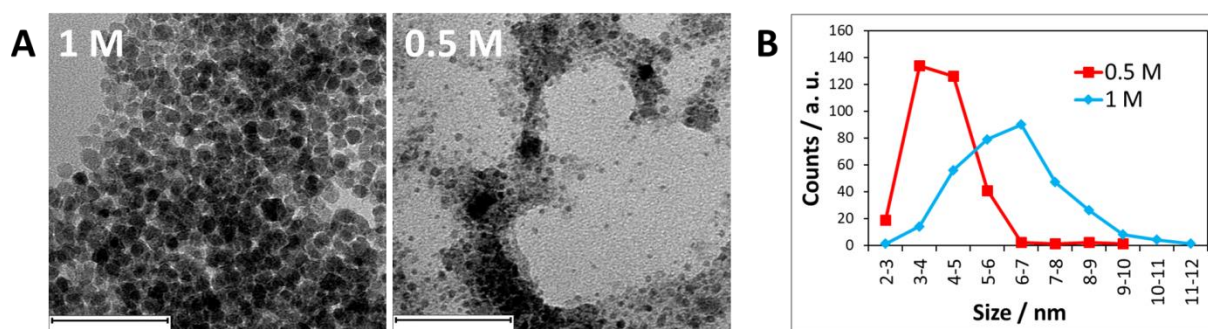


Figure 3 – TEM images (A) and size distributions (B) of USPIOs prepared from 1 M and 0.5 M precursor solutions. Scale bar is 50 nm.

References

- [1] Y. Lvov, W. Wang, L. Zhang, R. Fakhrullin, Halloysite Clay Nanotubes for Loading and Sustained Release of Functional Compounds, *Adv. Mater.* 28 (2016) 1227–1250. doi:10.1002/adma.201502341.
- [2] E. Abdullayev, V. Abbasov, A. Tursunbayeva, V. Portnov, H. Ibrahimov, G. Mukhtarova, Y. Lvov, Self-healing coatings based on halloysite clay polymer composites for protection of copper alloys, *ACS Appl. Mater. Interfaces.* 5 (2013) 4464–4471. doi:10.1021/am400936m.
- [3] E. Licandro, S. Cauteruccio, D. Dova, Thiahelices, in: 2016: pp. 1–46. doi:10.1016/bs.aihch.2015.12.001.
- [4] E. Joussein, S. Petit, J. Churchman, B. Theng, D. Righi, B. Delvaux, Halloysite clay minerals – a review, *Clay Miner.* 40 (2005) 383–426. doi:10.1180/0009855054040180.
- [5] M.R. Ayatollahi, S. Shadlou, M.M. Shokrieh, M. Chitsazzadeh, Effect of multi-walled carbon nanotube aspect ratio on mechanical and electrical properties of epoxy-based

nanocomposites, Polym. Test. 30 (2011) 548–556. doi:10.1016/j.polymertesting.2011.04.008.

- [6] I. Ksouri, O. De Almeida, N. Haddar, Long term ageing of polyamide 6 and polyamide 6 reinforced with 30% of glass fibers: physicochemical, mechanical and morphological characterization, J. Polym. Res. 24 (2017) 133. doi:10.1007/s10965-017-1292-6.
- [7] H. Shinzawa, J. Mizukado, Water absorption by polyamide (PA) 6 studied with two-trace two-dimensional (2T2D) near-infrared (NIR) correlation spectroscopy, J. Mol. Struct. 1217 (2020) 128389. doi:10.1016/j.molstruc.2020.128389.
- [8] K.R. Rajeesh, R. Gnanamoorthy, R. Velmurugan, Effect of humidity on the indentation hardness and flexural fatigue behavior of polyamide 6 nanocomposite, Mater. Sci. Eng. A. 527 (2010) 2826–2830. doi:10.1016/j.msea.2010.01.070.
- [9] D.P.N. Vlasveld, J. Groenewold, H.E.N. Bersee, S.J. Picken, Moisture absorption in polyamide-6 silicate nanocomposites and its influence on the mechanical properties, Polymer (Guildf). 46 (2005) 12567–12576. doi:10.1016/j.polymer.2005.10.096.
- [10] K.P. Pramoda, T. Liu, Effect of moisture on the dynamic mechanical relaxation of polyamide-6/clay nanocomposites, J. Polym. Sci. Part B Polym. Phys. 42 (2004) 1823–1830. doi:10.1002/polb.20061.
- [11] K. Prashantha, M.F. Lacrampe, P. Krawczak, Processing and characterization of halloysite nanotubes filled polypropylene nanocomposites based on a masterbatch route: effect of halloysites treatment on structural and mechanical properties, Express Polym. Lett. 5 (2011) 295–307. doi:10.3144/expresspolymlett.2011.30.
- [12] J.W. Goodwin, J. Hearn, C.C. Ho, R.H. Ottewill, Studies on the preparation and characterization of monodisperse polystyrene latices, Colloid Polym. Sci. 252 (1974) 464–471.
- [13] L. Rimoldi, V. Pifferi, D. Meroni, G. Soliveri, S. Ardizzone, L. Falciola, Three-dimensional mesoporous silica networks with improved diffusion and interference-abating properties for electrochemical sensing, Electrochim. Acta. 291 (2018) 73–83. doi:10.1016/j.electacta.2018.08.131.
- [14] J. Dulińska-Litewka, A. Łazarczyk, P. Hałubiec, O. Szafranski, K. Karnas, A. Karewicz, Superparamagnetic Iron Oxide Nanoparticles—Current and Prospective Medical Applications, Materials (Basel). 12 (2019) 617. doi:10.3390/ma12040617.
- [15] Y. Okuhata, Delivery of diagnostic agents for magnetic resonance imaging, Adv. Drug Deliv. Rev. 37 (1999) 121–137. doi:10.1016/S0169-409X(98)00103-3.
- [16] J.-C. Brisset, M. Sigovan, F. Chauveau, A. Riou, E. Devillard, V. Desestret, M. Touret, S. Nataf, J. Honnorat, E. Canet-Soulas, N. Nighoghossian, Y. Berthezene, M. Wiart, Quantification of Iron-Labeled Cells with Positive Contrast in Mouse Brains, Mol. Imaging Biol. 13 (2011) 672–678. doi:10.1007/s11307-010-0402-1.
- [17] M. Rogosnitzky, S. Branch, Gadolinium-based contrast agent toxicity: a review of known and proposed mechanisms, BioMetals. 29 (2016) 365–376. doi:10.1007/s10534-016-9931-7.
- [18] H. Wei, O.T. Bruns, M.G. Kaul, E.C. Hansen, M. Barch, A. Wiśniowska, O. Chen, Y. Chen, N. Li, S. Okada, J.M. Cordero, M. Heine, C.T. Farrar, D.M. Montana, G. Adam, H. Ittrich, A. Jasanoff, P. Nielsen, M.G. Bawendi, Exceedingly small iron oxide

nanoparticles as positive MRI contrast agents, *Proc. Natl. Acad. Sci.* 114 (2017) 2325–2330. doi:10.1073/pnas.1620145114.

- [19] Y. Bao, J.A. Sherwood, Z. Sun, Magnetic iron oxide nanoparticles as T₁ contrast agents for magnetic resonance imaging, *J. Mater. Chem. C*. 6 (2018) 1280–1290. doi:10.1039/C7TC05854C.

Oxides and their role in materials science

Nowadays, one of the main driving forces of technological advance is the development of materials with new and improved physicochemical properties, capable of performing an ever-increasing number of tasks, often at the same time. In the last decades, the research field of materials science has become an integral part of other scientific domains: Fields such as biology, medicine and even agriculture have started to rely more and more on the development of new functional materials.

One of the most versatile classes of materials is that of metal oxides, which shine for their abundance, chemical and mechanical stability, biocompatibility and tunability. Their eclectic physicochemical properties, such as their varied electrochemical behaviour and their easily modifiable surface properties, allow them to be adopted for a wide range of applications, both at research and industrial level: electrochemical sensors, drug nanocarriers, heterogeneous catalysts, photoelectrode materials in solar cells, and smart surfaces, to name a few.

In many of these applications, surface properties play a pivotal role, as interface phenomena regulate most interactions with the environment. A great number of oxidic systems developed today are nanosized, to increase their specific surface area, promote their reactivity and tailor their morphology, designing and making use of specific shapes, sizes and textures to achieve the desired effect. The high degree of control over the morphology of these materials is indeed one of their most relevant features.

On the same note, surface modification with functional molecules represents one of the best tools to enhance and modify the behaviour of oxides. Furthermore, the general abundance of hydroxyl groups and the high surface charge density make their functionalization, by forming either covalent or electrostatic bonds, relatively simple. Thanks to these factors, with the advent of a growing number of multifunctional oxide-based materials, many issues that could not be solved through molecular chemistry alone are being confronted with new resources.

Within this framework, the present thesis focuses on three groups of metal oxides: aluminosilicates, titanium dioxide and iron oxides.

As the most abundant family of minerals on Earth's crust, aluminosilicates have always been a staple of mankind's technological advancement, since the dawn of civilization, when they were used in pottery, glassware and construction, up until the development of modern materials science, where they see vast application in polymeric composites, catalysis and molecular separation. Their popularity stems from their excellent thermomechanical stability and peculiar electrochemical features, which earned them a paramount role in the chemical industry as either heterogeneous catalysts, or additives for the preparation of enhanced polymer composites and flame-retardant materials. More recently, their use in a multitude of additional fields has been investigated: their porous morphology has proven very useful for thermal energy storage, CO₂ capture and conversion, air-pollution remediation and water purification, while their innate biocompatibility has stimulated their application in the biomedical field, as diagnostic devices or drug delivery systems.

Titanium dioxide-based materials have played a central role both in our everyday life and in more advanced scientific contexts since the beginning of the twentieth century, when they started being adopted as pigment in paints instead of toxic lead oxides. From then on, their use has spread to countless other fields, from cosmetics to food packaging, from renewable energy to pollution remediation, just to name a few. Even though the stability and high refractive index of titanium dioxide are behind the initial success of these materials, the feature that fuelled the huge amount of research performed for over fifty years on TiO_2 is its semiconductive nature, which provides it with photocatalytic properties that make it the most diffused photocatalyst. Its photochemical behaviour has been applied to catalyse organic oxidation and degradation reactions, of water splitting and for energy production. Moreover, it is easily synthesised, its properties can be enhanced by doping and composite formation, its morphology can be readily tuned to produce a wide variety of different materials.

Finally, a third group of oxide-based materials that has deeply affected the modern world is represented by iron oxides. Their number is significant, counting sixteen different species, including oxides, hydroxides, and oxide-hydroxides, and their diffusion in our life is ubiquitous: they are applied as pigments in paints as well as in the plastic, steel and cosmetic industries. In the last few years, most of the attention has been devoted to two phases in particular, magnetite (Fe_3O_4) and maghemite ($\gamma\text{-Fe}_3\text{O}_4$), both characterized by a magnetic behaviour. This feature, traditionally exploited for the production of permanent magnets and recording tape, has recently found new uses in the preparation of data storage devices and biomedical applications. In this regard, iron oxide nanoparticles have been largely studied as drug delivery devices and MRI contrast agents thanks to their unconventional properties: in fact, below a certain size, generally 20-30 nm, their magnetic behaviour shifts from ferrimagnetic to superparamagnetic, a unique state that allows particles to behave like non-magnetic particles, freely rotating and diffusing in the dispersant, until they are subjected to an external field, which can orientate and guide them. Moreover, the paramagnetic Fe nuclei can affect relaxation times of hydrogen atoms in physiological water. These two features combined have driven the research on these systems in the field of theranostics, devices that unite therapy with diagnosis.

Although the innate properties of all these materials represent indeed an excellent starting base, the horizon of potential applications starts to expand dramatically once the element of surface modification is introduced. As mentioned above, oxide surfaces are relatively easy to modify, opening the door to new possibilities. For example, biocompatible molecules and polymers can be grafted on the surface of oxide nanoparticles to enhance their bioavailability, while the addition of peptizing agents can help with colloidal stability issues. Further, active molecules can be loaded on oxide-based materials with the aim of delivering them under specific conditions, through what is called “triggered release”.

In light of these considerations, this thesis will explore each of the abovementioned families of oxides, due to their relevance in modern science, and their modification aimed at developing multifunctional nanosystems. In particular, significant efforts have been devoted to the study of halloysite, a naturally nanotubular aluminosilicate, TiO_2 films with varied morphologies and Superparamagnetic Iron Oxide Nanoparticles (SPION), which have been synthesised and functionalised to serve a wide range of

purposes: among these, the development of selective therapeutic and diagnostic devices, the design of smart surfaces providing photo-regeneration and active principles release, and the production of improved oxide-based polymeric nanocomposites will be discussed in this thesis.

PART I

Halloysite nanotubes

INTRODUCTION

Halloysite represents an emerging nanoclay material with appealing properties. First described by Berthier in 1826 as a dioctahedral 1:1 clay mineral of the kaolin group, he gave it the name 'halloysite' in honour of Omalius d'Halloy who originally discovered it, after identifying it in a sample from Liège, Belgium. Nonetheless, halloysite started being extensively studied over 100 years later, in 1940, and only around 2000 it became available in pure form, being used as a low-cost alternative to carbon and boron nitride nanotubes due to its cylindrical shape.

It occurs largely in weathered rocks and soils[1]; it originated from the alteration of a wide range of both igneous and non-igneous rocks, although halloysite is often found as a major component of volcanic soils in wet tropical and subtropical regions. Halloysite particles can present a wide range of morphologies, the most common thereof is definitely the elongated tubule. However, short nanotubes, platelets and spheroidal particles have all been reported in the literature[2].

I.1 General properties

I.1.1 Structural properties

Mainly extracted from mines in China, Australia, Mexico and Brazil, halloysite is a hydrated polymorph of kaolinite, bearing the raw formula $\text{Al}_2(\text{OH})_4\text{Si}_2\text{O}_5 \cdot n\text{H}_2\text{O}$ [3]. The hydrated form, for which $n = 2$, is named "halloysite-(10 Å)", referring to the d_{001} value of the layers; this form envelopes one layer of water molecules between multilayers[4]. The dehydrated structure of halloysite, with $n = 0$, is named "halloysite-(7 Å)" and may be obtained through the loss of intercalated water under mild heating and/or a vacuum environment. The loss of interlayer water is quick and irreversible if the material is exposed to said conditions. Thus, halloysite-(10 Å) is more commonly found at depth, while halloysite-(7 Å) occurs near the soil surface, as upper soil layers are subject to desiccation during dry seasons. These factors suggest that halloysite-(10 Å) is a metastable species and halloysite-(7 Å) is the truly stable form, as further confirmed by the fact that once lost, interlayer water cannot normally be rehydrated by hydration treatments, making it an irreversible process.

As said before, while naturally occurring halloysite appears in a wide range of morphologies, the tubular structure represents its dominant state. This is because, as in kaolin, the crystalline lattice is constituted by layers of tetrahedral SiO_4 units and octahedral AlO_6 units; in halloysite, the mismatch between the larger tetrahedral sheets and the smaller octahedral ones causes the development of atomic-scale stress. Structural strain is solved by the interstitial disposition of water molecules that force kaolin sheets to wrap and assume a tubular shape in which the inner and outer surfaces differ from each other (Figure I.1). The latter is mainly composed of siloxane groups (Si-O-Si), even though silanol groups (Si-OH) are usually present as structure defects; the inner side is constituted of aluminium hydroxide $\text{Al}(\text{OH})_2$, showing a variety of different hydroxyls (monohydroxyls Al-OH , dihydroxyls, bridged hydroxyls).

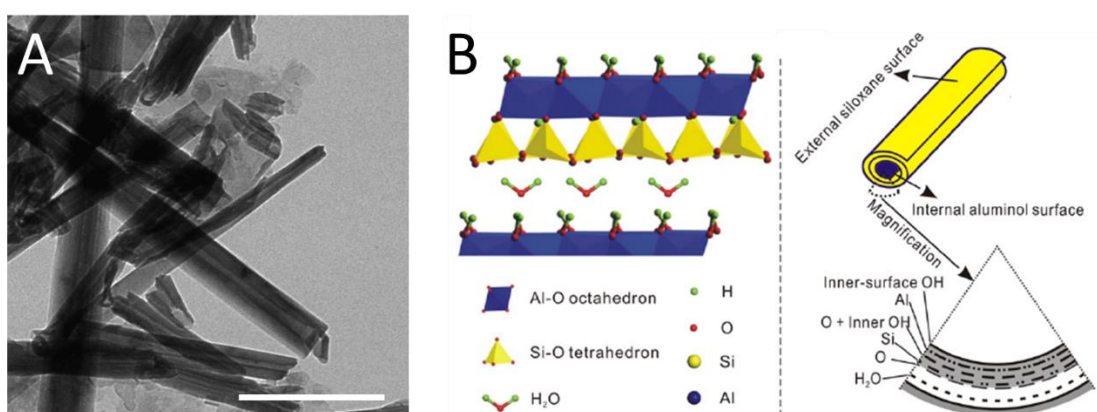


Figure I.1 - TEM image (A) and structural representation (B; Yuan et al., *App. Clay Sci.*, 112-113, 2015) of halloysite nanotubes. Scale bar is 500 nm.

I.1.1 Morphological features

The morphological parameters of halloysite (length, diameter, aspect ratio, and tube thickness) vary significantly between and even within deposits[4], and such diversity is due to its formation under various crystallization conditions and geological occurrences. Moreover, several studies report a close connection between the amount of Fe^{3+} impurities in the crystalline lattice and the adopted morphology[1,5,6]: it seems that the extent of the isomorphous substitution of Al^{3+} with Fe^{3+} ions dictates the curvature degree of rolled kaolin sheets, which straighten as the iron amount increases. This trend is confirmed by the fact that, among all potential halloysite morphologies, platelets and spheroidal particles show the highest Fe^{3+} content, while nanotubes have the lowest. The same substitution effects which define the final morphology of halloysite formations, can also influence the morphological parameters within the same particle category, for example the length of nanotubes. The same trend observed in the case of layer curvature regulates the nanotubes aspect ratio: as structural Fe content increases, the average length and aspect ratio of the particles decrease. A possible explanation of such behaviour is that, during halloysite crystallization, Fe ions act as nucleation sites: a small iron concentration would favour the growth of long tubes, while appreciable

amounts would encourage nucleation, leading to shorter tubes[7]. For these reasons, their length ranges from 100 nm to even 4 μm , while their diameter measures few tens of nanometres. Halloysite nanotubes possess relatively high specific surface area (SSA) and total pore volume, due to the abundance of pores in their structure. They can be divided into three main categories: 1) the slit-shaped longitudinal mesopores generated during the dehydration process by the separation of the formerly tightly connected layers; 2) the inherently mesoscopic, or even macroscopic, lumina (the inner cavity of the tubular halloysite particle); 3) the voids formed among particles in agglomerates (50–100 nm). The variability of halloysite porosity, even for samples obtained from the same extraction site, is significant, both because of its different morphologies and the presence of various associated minerals.

I.1.1 Surface charge

Both halloysite surfaces, when immersed in water at pH values between 2 and 8, are charged. The external silica bears a negative charge, while the inner alumina phase is positive, resulting in an average electrical ζ -potential value of around -40 mV. This value can change based on the specific morphology of the halloysite under study, as the cation exchange capacity (CEC), and consequentially surface charge density, increase with the structural disorder of the mineral. The isomorphic substitution of Si by Al induces the formation of some permanent negatively charged surface sites XO_2^- , such as $(\text{Al-O-Si})_2^-$, on the siloxane layer, which are responsible for the major part of surface ion exchange reactions[8]. Charge separation is closely linked to the acid/base properties of HNT: Bretti et al. studied the protonation and deprotonation equilibria of Si-OH and Al-OH groups, using potentiometric titrations under different conditions of pH, ionic medium, ionic strength and particle concentration[9]. Different protonation constants were defined for halloysite, one for Si-OH groups and two for Al-OH groups, and all of them increased with ionic strength in all ionic media. This suggests that protonated species are stabilized by the formation of weak complexes with the ions of a background electrolyte (Figure I.2).

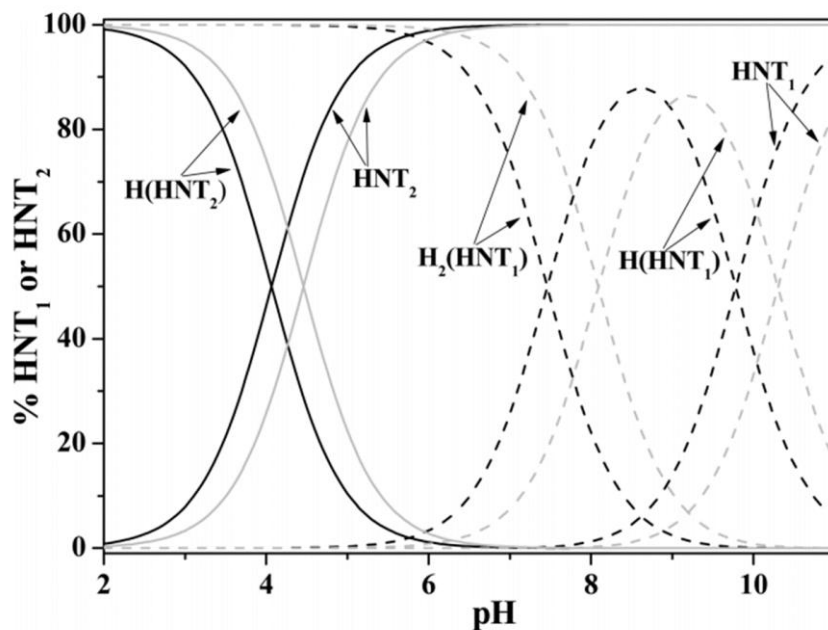


Figure I.2 - Distribution diagrams of surface AlOH (HNT_1) and SiOH (HNT_2) and derived species, plotted against pH. (Bretti et al., *J. Phys. Chem. C*, 120, 2016)

I.1.1 Biocompatibility

Halloysite biocompatibility has been demonstrated in a series of literature reports, which studied HNT effect on cell proliferation and biological functions of complex organisms. Cellular uptake and cytotoxicity have been tested on human tumour cell lines, including thyroid, hepatic and breast cancer cells[3,10,11], proving that it is non-toxic up to a concentration of 0.1 mg mL^{-1} . Halloysite effects on living organisms have been assessed through an *in vivo* study on *Caenorhabditis elegans*, a free-living nematode worm[12]: it was demonstrated that halloysite nanotubes were exclusively located in the worms intestines and in no other body part of nematodes. This suggests that, over a wide concentration range, halloysite does not harm living organisms, as it just caused mechanical stress on the nematode digestive system.

Halloysite accumulation in the body is prevented by its removal at the hand of macrophages, confirming its biocompatible nature, although cellular uptake has been confirmed by confocal microscopy. Nonetheless, phagocytised HNT did not prevent cells from proliferating, even though nanotubes were found to accumulate around the cell nucleus: this surprising proximity to the nucleus could be exploited to deliver important bioactive agents directly to the most important part of cells, as demonstrated by fluorescence microscopy[3]: the functionalization of halloysite surface with targeting moieties could lead to a cellular uptake concentrated around the nucleus, especially when nanotubes were covered with sugar moieties, which provided a high propensity to cross the cell lipid membranes.

I.2 Applications

All the abovementioned properties make halloysite an extremely versatile material, with a vast horizon of potential applications. Halloysite is among the rare examples of nanotubes with a different composition of inner and outer surfaces. Most of the reported non-clay nanotubes, such as carbon nanotubes and boron nitride nanotubes, have the same inner and outer chemistry, which makes their selective lumen modification difficult. The fairly unique structure of halloysite can be exploited to modify separately the two surfaces, making them able to perform different tasks. Together with their dual nature, HNT biocompatibility, low cost and good dispersibility in physiological conditions (even without any surface modification) make them suitable as integral components of biocompatible materials. Furthermore, they show a moderate porosity and surface area, increasing their loading capabilities. Thanks to these characteristics, halloysite nanotubes sparked interest in the biomedical field, in particular as biocompatible drug carriers, and in numerous other research fields: their potential uses are many and varied, ranging from adsorbants for polluting agents to additives in polymers, to which they confer mechanical and thermal stability. In the following

sections, the main applications investigated in the present thesis will be briefly presented.

I.2.1 Nanomedical application

One of the most interesting fields of application is indeed nanomedicine, where halloysite peculiar morphology and low cytotoxicity can be exploited to deliver therapeutic and diagnostic agents inside the organism.

During the last twenty years, a great deal of effort has been devoted to developing efficient drug carriers based on the surface modification of a wide range of scaffolds. To be effective in its task, a drug delivery device must possess a series of characteristics that often exceed what can be reasonably expected from a single, unmodified material. For this reason, success in designing drug carriers is almost always the result of a finely tuned combination between the choice of an eclectic, multifunctional scaffold and a tailored surface modification procedure.

The characteristics of the starting material, acting as carrier for the administration of drugs and treatments to living organisms, must comply with more and stricter requisites with respect to any other application field. Hence, the chosen material should ideally be:

- Non-cytotoxic, in order not to cause indiscriminate cellular apoptosis when introduced in the body;
- Able to cross the lipid membranes of cells, to deliver bioactive compounds directly to the cytoplasm or the nucleus;
- Biodegradable or at least be subject to physiological removal pathways, to avoid accumulation in the organism;
- Well dispersed under physiological conditions, as to avoid aggregation and the consequential formation of blood clots;
- Easily modifiable, to facilitate surface functionalization with functional molecules, such as linkers and drugs[13].

Surface functionalization is indeed one of the key aspects of drug carrier design and refers to the introduction of functional groups and bioactive molecules onto the surface of the host material, sometimes radically changing or enhancing its original properties. It can be achieved by either physical interactions, anchoring the functionalizing molecule onto the scaffold by Van der Waals forces, hydrogen bonding or electrostatic attraction, or chemical modifications, covalently binding the molecule of interest to the host. As said before, this procedure is extremely useful to alter both the physical properties, such as solubility, dispersibility, wettability, and the chemical features, like surface reactivity and cytotoxicity, of the host. This allows for the careful tailoring of the device behaviour, which can be optimized for specific applications.

In this regard, halloysite nanotubes possess most, if not all, of the abovementioned features: they are reportedly biocompatible, are subject to cellular uptake through endocytosis[14] and to elimination by macrophages[3], their high surface charge provides a good dispersibility in physiological environments and, lastly, both their surfaces are easily modifiable thanks to Si-OH and Al-OH groups.

For these reasons, numerous studies[15–17] have been directed at the loading of Active Pharmaceutical Ingredients (API) on HNT, focusing both on physisorbed and chemisorbed molecules. Although physisorption is easier to achieve, thanks to the dually-charged surface allowing for electrostatic interactions with both cations and anions, the release rates depend completely on diffusion, which normally is unspecific and time dependent, reducing targeting efficiency. Moreover, an initial release burst is often observed in the case of non-covalently loaded molecules, which may compromise the targeting ability and shelf-life of these systems, precluding their preservation as suspensions to avoid premature drug diffusion.

Most of the studies regarding compounds physisorbed on HNT exploit biocompatible polymers such as chitosan[18] and PEG[19], using them as a coating capable of housing therapeutic drugs like Doxorubicin, or biological macromolecules such as proteins and nucleic acids. The external siloxane surface of halloysite has a lower chemical activity and is scarcely available for grafting by organic compounds. However, due to the negative surface potential in a wide pH range, the external surface properties of HNT can be modified by adsorbing some specific cations[20] or even DNA strains[21]. Furthermore, the 10–20 nm inner diameters of halloysite tubes make them a good container for loading globular proteins with diameters of 3–8 nm. Besides, the positive charge of aluminium hydroxides makes it possible to utilize electrostatic interactions to enhance internal loading in the tubes, preventing external protein adsorption. Proteins at a pH above their isoelectric point are predominantly negatively charged, and can thus be efficiently loaded inside the tubes.

To improve HNT loading capabilities, etching pre-treatments of the nanotubes can also be used. In highly concentrated acid or alkaline solutions, the structure of HNT undergoes significant changes. White et al. conducted a detailed study of the long-term stability of natural HNT under strong acidic and alkaline conditions[22]. Both the strong acid and alkaline treatments led to an increase in both specific surface area and pore volume compared to the original HNT, due to the enlargement of the inner lumen and shrinking of the tube walls. This kind of morphological modification, without any significant shape alteration after the acid or alkaline treatment, can be desirable for applications of modified HNT where a larger internal diameter is required, such as the encapsulation of large proteins or DNA molecules.

On the other hand, chemisorption is harder to perform and often overlooked in favour of electrostatic interaction, as the reactivity of many organic molecules is not entirely compatible with that of aluminosilicates and it often requires more complicated reaction conditions. Nonetheless, if properly chosen covalent bonds are used, their cleavage and subsequent drug release can be more selectively controlled by specific physiological conditions, effectively producing stimuli-responsive drug delivery devices.

Drug release can be elicited by different environmental factors, including pH variations, the action of cellular enzymes, or temperature changes. In this context, one of the most investigated classes of functionalizing agents is represented by responsive polymers, that show conformational changes upon exposition to an external stimulus. In a 2015 study on a release system sensitive to temperature, obtained by coating the surface of halloysite with a thermo-responsive polymer, poly-N-isopropyl acrylamide (PNIPAAm)[23]. The polymer, attached via amide condensation with a previously

grafted linker, undergoes a coil-to-globule transition at around 32°C: the drug, curcumin, was loaded onto the carrier at 25 °C and release was tested *in vitro* at 37 °C, in a medium simulating the conditions of the human gastro-intestinal tract, confirming the sustained release of the active species. While interesting, temperature-responsive systems still do not address the issue of selective targeting, as body temperature is not subject to significant variations between different tissues.

Among the reported covalently-loaded HNT, many research works have focused on surface modification and successive drug loading using unselective functionalizing agents, such as organosilanes like (3-aminopropyl)triethoxysilane (APTES)[24–26], which can bind to both the silica and alumina surfaces. A possible alternative has been reported by Takahara et al. in a 2012 study[27], in which 2-bromo-N-[2-(3,4-dihydroxyphenyl)ethyl]-isobutyryl amide, a dopamine derivative, was immobilized onto the internal lumen surface of HNT. This compound is expected to bind to both the silica-like external surface and the alumina-like internal lumen surface of halloysite through silica–catechol bonds and alumina–catechol bonds, respectively. However, the authors reported that the weak silica–catechol bonds could be broken by sonication and tetrahydrofuran–water solution rinses, which did not affect the more stable alumina–catechol bonds.

The dual nature of HNT inner and outer surfaces provides a means for a selective functionalization and loading of the two surfaces. If implemented, such a feature could open the doors to a series of additional functional behaviours, which rely on a combination of different effects to perform complex tasks. For this reason, the surface-selective modification of HNT deserves further attention, as it could make HNT particularly suited for advanced applications, such as in theranostics. Theranostic agents, *i.e.* systems combining illness detection and treatment, have recently become a hot topic in nanomedicine: these systems can be seen as nano-sized envelopes, carrying both therapeutic and diagnostic compounds, capable of reaching the target tissue, highlighting it for specific imaging techniques and releasing the active ingredient to treat the disease. This is one of the main directions recently taken by the research on Super Paramagnetic Iron Oxide Nanoparticles (SPION), gold nanoparticles and other inorganic systems. Along these lines, some studies have investigated HNT as probes for diagnostics purposes, taking advantage of grafted photoluminescent molecules[28,29] or their ability to backscatter ultrasounds[30] thus enhancing echographic imaging. Those diagnostic approaches can be coupled with the loading of bioactive molecules for the *in situ* release, achieving a theranostic behaviour.

I.2.2 Polymeric composites

As numerous other nanometric clay minerals[31–33], halloysite has also been considered as a potential filler in polymeric nanocomposites, to enhance their thermomechanical properties and provide additional interesting functionalities, such as improved durability, flame-retardant behaviour and low-permeability membrane properties.

As the components in nanocomposites, namely the filler and polymer matrix, often possess contrasting surface free energy and physicochemical properties, the effectiveness of nanoparticles incorporation as fillers mainly depends on:

- 1) The filler aspect ratio and surface area, which are fundamental to guarantee stronger interactions with the polymer matrix: morphologies with high aspect ratio, such as nanotubes, are thus favoured;
- 2) Homogeneous dispersion of the filler within the matrix, without the formation of agglomerates.

The last point is especially important, as increased dispersion helps to achieve uniform stress distribution and improved resistance to infiltrations in the matrix. For this reason, reaching such a dispersion degree to the point where each individual particle is coated by polymer chains is extremely critical. Due to Van der Waals forces and electrostatic interactions, nanofillers usually tend to aggregate during their incorporation in the polymer. Although Van der Waals interactions are considered weak forces, the large specific surface area of nanomaterials turns them in a significant factor, often leading to particles agglomerates. One of the main issues with one of the most investigated nanotubular fillers, carbon nanotubes, is that they tend to self-assemble along the length axis into rope-like structures that slip when put under mechanical stress, due to their poor amalgamation with the polymer matrix, which negatively affects the composite properties[34]. Nanotube bundles can act as stress and moisture concentration points in the material, in some cases even worsening the mechanical properties and water resistance of the pristine polymer.

Low compatibility between polymer and filler is usually compensated by the increased interfacial area available for nanosized materials. A strong bonding between filler and matrix is essential to reinforce the nanocomposite and avoid filler aggregation, which leads to the stability issues discussed before. When the interfacial area is not enough to provide such bonding, nanoparticles can be functionalized with organic compounds to increase their dispersibility and enhance interaction with the polymeric matrix. Anisotropic fillers, such as platelets and nanotubes, can also align when dispersed: while alignment is not paramount, it can have a positive effect on the strength of the final nanocomposite.

In this framework, the positive aspects associated with the use of halloysite nanotubes as fillers are multiple:

- HNT are abundant and low-cost, which is a crucial aspect from an industrial point of view;
- They have a high aspect ratio and surface charge, which improve filler dispersion in the matrix, hindering the formation of aggregates. Additionally, their predominantly negative surface charge facilitates electrostatic attractions with cationic polymers, which is particularly useful when designing the interface of certain polymer nanocomposites: for example, the layer-by-layer (LbL) assembly can be adopted to prepare finely layered thin films[20];
- They have high mechanical strength, which makes them suitable for stress-intensive applications. A variety of polymers can benefit from the addition of halloysite, even at low loadings, as it simultaneously improves the strength, modulus, stiffness and impact resistance[35,36]. Additionally, these enhancements are noticeable even at high temperatures, under both dynamic and static conditions[37];

- They are natural, halogen-free flame retardants due to their thermal stability, acting as barriers against heat and mass transport during the combustion of nanocomposites[38]; furthermore, their tubular morphology causes the entrapment of polymer decomposition products in the lumen and the presence of iron impurities decreases the general flammability of the composite, thanks to the ability of Fe atoms to trap radicals produced during thermal degradation[39]. Their marked surface acidity has also been reported to promote char formation[40];
- They have two different easily modifiable surfaces, opening the possibility to improve their incorporation in the polymer while still being able to carry functional compounds. In this respect, it should be noted that, as interparticle interactions are relatively weak, they reach a good dispersion degree by shear during processing; surface functionalization is thus not necessary, but it greatly improves their incorporation in the polymer matrix;
- They are biocompatible, allowing for the preparation of bionanocomposites applicable in tissue engineering scaffolds, drug delivery, cancer cell isolation, bone implant and cosmetics; They act as nucleating agents, shortening processing time for crystallized polymers while enhancing the end product. Adding a small amount of halloysite can significantly increase crystallization rates for slow-crystallizing polymers such as polylactide, shortening an otherwise lengthy processing period[41]. Their addition, even at high loadings, only partially affects the natural viscosity of polymer melt and solutions. Rheological tests proved that shear viscosity was not significantly affected up to 30% w/w halloysite, and even then nanocomposites could still be processed under similar conditions as the pristine polymer[42].

All these characteristics make them a cheaper, biocompatible alternative to the more expensive and less biocompatible carbon and boron nitride nanotubes, with all the advantages of tubular nanosystems over other clay fillers, like superior aspect ratio and one-dimensionality.

For these reasons, in the last decade, much effort has been dedicated to the study of halloysite-polymer nanocomposites as flame-retardant materials[43,44], tissue engineering scaffolds[45], bone implants[46] and even cosmetics[47]. A 2013 study reported the preparation of a composite based on polyamide-11, showing how a good incorporation of halloysite in the matrix lead to an increase in tensile strength and Young's modulus without sacrificing the ductility of the polymer[48]. Moreover, the addition of HNT as fillers reportedly brought favourable changes in thermal and rheological properties of the pristine material, increasing glass transition temperature, crystallization temperature, and degree of crystallinity of the nanocomposites. In a more recent study, the effect of halloysite nanotubes on the mechanical properties of low-density polyethylene composites was tested, incorporating a variable amount of filler (from 2% to 6% w/w)[49]. It was found that the presence of halloysite brought to a significant heat deflection temperature increase, up to 47°C, also improving the tensile strength of the composites. On the other hand, the composite processability, defined by the melt flow rate, was negatively affected by the addition of nanotubes, but only when they were not well dispersed in the polymer matrix

Thanks to the demonstrated biocompatibility of this material, several studies reported in the literature focused on the addition of halloysite nanotubes to a biopolymer matrix. Pristine biopolymer matrices suffer from intrinsic disadvantages, including burst release of drugs and low stability. To solve these issues, inorganic fillers have been reportedly adopted to improve, other than mechanical properties, also the swelling behaviour, drug loading and release control of biopolymer matrices through electrostatic interactions and hydrogen bonding. In this context, the combination of halloysite nanotubes with biopolymers is a feasible approach to prepare innovative medical materials for sustained drug release. A 2014 study reported the addition of halloysite to a chitosan matrix[50]. Thanks to its haemostatic and antimicrobial properties, chitosan can be exploited to hasten the healing process of wounds, as it accelerates the infiltration of polymorphonuclear cells in the early stages of wound healing, encouraging the production of collagen at the hand of fibroblasts. The study clearly showed that the addition of halloysite nanotubes almost doubled blood clotting rates with respect to pure chitosan, aiding in re-epithelialization and collagen deposition, demonstrating the potential application of HNT-chitosan nanocomposite sponges for diabetic, burn and chronic wound infections. In another study, halloysite nanotubes functionalized with cucurbit[6]uril molecules were loaded with essential peppermint oil, known to have antimicrobial properties, and added to a pectin polymer matrix to prepare edible biodegradable films for food packaging applications[51]. This biocomposite film was proven to be more effective at higher temperatures, suggesting its potential use as packaging for precooked food products. Chen et al. published their work on the preparation of halloysite-reinforced polylactic acid (PLA), with potential medical applications in instances that require high mechanical strength, like bone fixation devices and coronary stents[52]. To this purpose, various weight fractions of halloysite nanotubes were added to the biodegradable PLA matrix, providing improved Young's modulus and stiffness, without affecting the wettability of the original polymer.

Following these premises, Chapters 1, 2 and 3 will delve into the study of the physicochemical properties of halloysite nanotubes and their surface modification with the purpose of employing them for diverse applications, including targeted drug delivery and moisture-resistant polymeric nanocomposites.

References

- [1] M.E. Sumner, Handbook of Soil Science, CRC Press, Boca Raton, Florida, 2000.
- [2] A. Singer, M. Zarei, F.M. Lange, K. Stahr, Halloysite characteristics and formation in the northern Golan Heights, *Geoderma*. 123 (2004) 279–295. doi:10.1016/j.geoderma.2004.02.012.
- [3] Y. Lvov, W. Wang, L. Zhang, R. Fakhrullin, Halloysite Clay Nanotubes for Loading and Sustained Release of Functional Compounds, *Adv. Mater.* 28 (2016) 1227–1250. doi:10.1002/adma.201502341.
- [4] E. Joussein, S. Petit, J. Churchman, B. Theng, D. Righi, B. Delvaux, Halloysite clay minerals — a review, *Clay Miner.* 40 (2005) 383–426.

doi:10.1180/0009855054040180.

- [5] R.D. Hart, R.J. Gilkes, S. Siradz, B. Singh, The Nature of Soil Kaolins from Indonesia and Western Australia, *Clays Clay Miner.* 50 (2002) 198–207. doi:10.1346/000986002760832793.
- [6] R.A. Eggleton, Hisingerite: A Ferric Kaolin Mineral with Curved Morphology, *Clays Clay Miner.* 46 (1998) 400–413. doi:10.1346/CCMN.1998.0460404.
- [7] G.J. Churchman, B.K.G. Theng, Interactions of halloysites with amides: mineralogical factors affecting complex formation, *Clay Miner.* 19 (1984) 161–175. doi:10.1180/claymin.1984.019.2.04.
- [8] G. Tari, I. Bobos, C.S.F. Gomes, J.M.F. Ferreira, Modification of surface charge properties during kaolinite to halloysite-7Å transformation, *J. Colloid Interface Sci.* 210 (1999) 360–366. doi:10.1006/jcis.1998.5917.
- [9] C. Bretti, S. Cataldo, A. Gianguzza, G. Lando, G. Lazzara, A. Pettignano, S. Sammartano, Thermodynamics of Proton Binding of Halloysite Nanotubes, *J. Phys. Chem. C.* 120 (2016) 7849–7859. doi:10.1021/acs.jpcc.6b01127.
- [10] M. Massaro, S. Piana, C.G. Colletti, R. Noto, S. Riela, C. Baiamonte, C. Giordano, G. Pizzolanti, G. Cavallaro, S. Milioto, G. Lazzara, Multicavity halloysite–amphiphilic cyclodextrin hybrids for co-delivery of natural drugs into thyroid cancer cells, *J. Mater. Chem. B.* 3 (2015) 4074–4081. doi:10.1039/C5TB00564G.
- [11] M. Massaro, R. Amorati, G. Cavallaro, S. Guernelli, G. Lazzara, S. Milioto, R. Noto, P. Poma, S. Riela, Direct chemical grafted curcumin on halloysite nanotubes as dual-responsive prodrug for pharmacological applications, *Colloids Surfaces B Biointerfaces.* 140 (2016) 505–513. doi:10.1016/j.colsurfb.2016.01.025.
- [12] G.I. Fakhrullina, F.S. Akhatova, Y.M. Lvov, R.F. Fakhrullin, Toxicity of halloysite clay nanotubes in vivo: a *Caenorhabditis elegans* study, *Environ. Sci. Nano.* 2 (2015) 54–59. doi:10.1039/C4EN00135D.
- [13] M.J. Mitchell, M.M. Billingsley, R.M. Haley, M.E. Wechsler, N.A. Peppas, R. Langer, Engineering precision nanoparticles for drug delivery, *Nat. Rev. Drug Discov.* (2020). doi:10.1038/s41573-020-0090-8.
- [14] H. Liu, Z.-G. Wang, S.-L. Liu, X. Yao, Y. Chen, S. Shen, Y. Wu, W. Tian, Intracellular pathway of halloysite nanotubes: potential application for antitumor drug delivery, *J. Mater. Sci.* 54 (2019) 693–704. doi:10.1007/s10853-018-2775-5.
- [15] A. Vikulina, D. Voronin, R. Fakhrullin, V. Vinokurov, D. Volodkin, Naturally derived nano- and micro-drug delivery vehicles: halloysite, vaterite and nanocellulose, *New J. Chem.* 44 (2020) 5638–5655. doi:10.1039/C9NJ06470B.
- [16] M.Y. Saleh, N. Prajapati, M.A. DeCoster, Y. Lvov, Tagged Halloysite Nanotubes as a Carrier for Intercellular Delivery in Brain Microvascular Endothelium, *Front. Bioeng. Biotechnol.* 8 (2020). doi:10.3389/fbioe.2020.00451.
- [17] Lin Sun, D.K. Mills, Halloysite nanotube-based drug delivery system for treating osteosarcoma, in: 2014 36th Annu. Int. Conf. IEEE Eng. Med. Biol. Soc., IEEE, 2014: pp. 2920–2923. doi:10.1109/EMBC.2014.6944234.
- [18] M. Liu, Y. Chang, J. Yang, Y. You, R. He, T. Chen, C. Zhou, Functionalized halloysite nanotube by chitosan grafting for drug delivery of curcumin to achieve enhanced

anticancer efficacy, *J. Mater. Chem. B.* 4 (2016) 2253–2263. doi:10.1039/C5TB02725J.

- [19] M. Di Paola, A. Quarta, P. Pisani, F. Conversano, E.A. Sbenaglia, S. Leporatti, G. Gigli, S. Casciaro, Surface Coating Highly Improves Cytocompatibility of Halloysite Nanotubes: A Metabolic and Ultrastructural Study, *IEEE Trans. Nanotechnol.* 15 (2016) 770–774. doi:10.1109/TNANO.2016.2546955.
- [20] Y. Lvov, R. Price, B. Gaber, I. Ichinose, Thin film nanofabrication via layer-by-layer adsorption of tubule halloysite, spherical silica, proteins and polycations, *Colloids Surfaces A Physicochem. Eng. Asp.* 198–200 (2002) 375–382. doi:10.1016/S0927-7757(01)00970-0.
- [21] M.H. Shamsi, K.E. Geckeler, The first biopolymer-wrapped non-carbon nanotubes, *Nanotechnology.* 19 (2008) 075604. doi:10.1088/0957-4484/19/7/075604.
- [22] R.D. White, D. V Bavykin, F.C. Walsh, The stability of halloysite nanotubes in acidic and alkaline aqueous suspensions, *Nanotechnology.* 23 (2012) 065705. doi:10.1088/0957-4484/23/6/065705.
- [23] G. Cavallaro, G. Lazzara, M. Massaro, S. Milioto, R. Noto, F. Parisi, S. Riela, Biocompatible Poly(N-isopropylacrylamide)-halloysite Nanotubes for Thermoresponsive Curcumin Release, *J. Phys. Chem. C.* 119 (2015) 8944–8951. doi:10.1021/acs.jpcc.5b00991.
- [24] M. Massaro, G. Lazzara, S. Milioto, R. Noto, S. Riela, Covalently modified halloysite clay nanotubes: synthesis, properties, biological and medical applications, *J. Mater. Chem. B.* 5 (2017) 2867–2882. doi:10.1039/c7tb00316a.
- [25] S. Leporatti, Halloysite clay nanotubes as nano-bazookas for drug delivery, *Polym. Int.* 66 (2017) 1111–1118. doi:10.1002/pi.5347.
- [26] P. Yuan, P.D. Southon, Z. Liu, M.E.R. Green, J.M. Hook, S.J. Antill, C.J. Kepert, Functionalization of halloysite clay nanotubes by grafting with γ -aminopropyltriethoxysilane, *J. Phys. Chem. C.* 112 (2008) 15742–15751. doi:10.1021/jp805657t.
- [27] W.O. Yah, H. Xu, H. Soejima, W. Ma, Y. Lvov, A. Takahara, Biomimetic Dopamine Derivative for Selective Polymer Modification of Halloysite Nanotube Lumen, *J. Am. Chem. Soc.* 134 (2012) 12134–12137. doi:10.1021/ja303340f.
- [28] V. Vergaro, E. Abdullayev, Y.M. Lvov, A. Zeitoun, R. Cingolani, R. Rinaldi, S. Leporatti, Cytocompatibility and uptake of halloysite clay nanotubes, *Biomacromolecules.* 11 (2010) 820–826. doi:10.1021/bm9014446.
- [29] H. Zhang, T. Ren, Y. Ji, L. Han, Y. Wu, H. Song, L. Bai, X. Ba, Selective Modification of Halloysite Nanotubes with 1-Pyrenylboronic Acid: A Novel Fluorescence Probe with Highly Selective and Sensitive Response to Hyperoxide, *ACS Appl. Mater. Interfaces.* 7 (2015) 23805–23811. doi:10.1021/acsami.5b08600.
- [30] G. Soloperto, F. Conversano, A. Greco, E. Casciaro, A. Ragusa, S. Leporatti, A. Lay-Ekuakille, S. Casciaro, Multiparametric evaluation of the acoustic behavior of halloysite nanotubes for medical echographic image enhancement, *IEEE Trans. Instrum. Meas.* 63 (2014) 1423–1430. doi:10.1109/TIM.2013.2287797.
- [31] Q. Dong, J. Yuan, X. Chen, X. Ma, Reduction of moisture susceptibility of cold asphalt

mixture with Portland cement and bentonite nanoclay additives, *J. Clean. Prod.* 176 (2018) 320–328. doi:10.1016/j.jclepro.2017.12.163.

- [32] L.A. Savas, M. Hancer, Montmorillonite reinforced polymer nanocomposite antibacterial film, *Appl. Clay Sci.* 108 (2015) 40–44. doi:10.1016/j.clay.2015.02.021.
- [33] S. Jafarzadeh, A.K. Alias, F. Ariffin, S. Mahmud, A. Najafi, Preparation and characterization of bionanocomposite films reinforced with nano kaolin, *J. Food Sci. Technol.* 53 (2016) 1111–1119. doi:10.1007/s13197-015-2017-7.
- [34] J.N. Coleman, U. Khan, W.J. Blau, Y.K. Gun'ko, Small but strong: A review of the mechanical properties of carbon nanotube–polymer composites, *Carbon N. Y.* 44 (2006) 1624–1652. doi:10.1016/j.carbon.2006.02.038.
- [35] V. Khunova, J. Kristof, I. Kelnar, J. Dybal, The effect of halloysite modification combined with in situ matrix modifications on the structure and properties of polypropylene/halloysite nanocomposites, *Express Polym. Lett.* 7 (2013) 471–479. doi:10.3144/expresspolymlett.2013.43.
- [36] U.A. Handge, K. Hedicke-Höchstötter, V. Altstädt, Composites of polyamide 6 and silicate nanotubes of the mineral halloysite: Influence of molecular weight on thermal, mechanical and rheological properties, *Polymer (Guildf).* 51 (2010) 2690–2699. doi:10.1016/j.polymer.2010.04.041.
- [37] M. Liu, B. Guo, M. Du, X. Cai, D. Jia, Properties of halloysite nanotube–epoxy resin hybrids and the interfacial reactions in the systems, *Nanotechnology.* 18 (2007) 455703. doi:10.1088/0957-4484/18/45/455703.
- [38] E.S. Goda, K.R. Yoon, S.H. El-sayed, S.E. Hong, Halloysite nanotubes as smart flame retardant and economic reinforcing materials: A review, *Thermochim. Acta.* 669 (2018) 173–184. doi:10.1016/j.tca.2018.09.017.
- [39] M. Du, B. Guo, D. Jia, Thermal stability and flame retardant effects of halloysite nanotubes on poly(propylene), *Eur. Polym. J.* 42 (2006) 1362–1369. doi:10.1016/j.eurpolymj.2005.12.006.
- [40] H. Qin, S. Zhang, C. Zhao, G. Hu, M. Yang, Flame retardant mechanism of polymer/clay nanocomposites based on polypropylene, *Polymer (Guildf).* 46 (2005) 8386–8395. doi:10.1016/j.polymer.2005.07.019.
- [41] M. Liu, Y. Zhang, C. Zhou, Nanocomposites of halloysite and polylactide, *Appl. Clay Sci.* 75–76 (2013) 52–59. doi:10.1016/j.clay.2013.02.019.
- [42] U.A. Handge, K. Hedicke-Höchstötter, V. Altstädt, Composites of polyamide 6 and silicate nanotubes of the mineral halloysite: Influence of molecular weight on thermal, mechanical and rheological properties, *Polymer (Guildf).* 51 (2010) 2690–2699. doi:10.1016/j.polymer.2010.04.041.
- [43] Z. Jia, Y. Luo, B. Guo, B. Yang, M. Du, D. Jia, Reinforcing and Flame-Retardant Effects of Halloysite Nanotubes on LLDPE, *Polym. Plast. Technol. Eng.* 48 (2009) 607–613. doi:10.1080/03602550902824440.
- [44] D.C.O. Marney, W. Yang, L.J. Russell, S.Z. Shen, T. Nguyen, Q. Yuan, R. Varley, S. Li, Phosphorus intercalation of halloysite nanotubes for enhanced fire properties of polyamide 6, *Polym. Adv. Technol.* 23 (2012) 1564–1571. doi:10.1002/pat.3030.
- [45] M. Liu, C. Wu, Y. Jiao, S. Xiong, C. Zhou, Chitosan–halloysite nanotubes

nanocomposite scaffolds for tissue engineering, *J. Mater. Chem. B.* 1 (2013) 2078. doi:10.1039/c3tb20084a.

- [46] W. Wei, E. Abdullayev, A. Hollister, D. Mills, Y.M. Lvov, Clay Nanotube/Poly(methyl methacrylate) Bone Cement Composites with Sustained Antibiotic Release, *Macromol. Mater. Eng.* 297 (2012) 645–653. doi:10.1002/mame.201100309.
- [47] Y.J. Suh, D.S. Kil, K.S. Chung, E. Abdullayev, Y.M. Lvov, D. Mongayt, Natural Nanocontainer for the Controlled Delivery of Glycerol as a Moisturizing Agent, *J. Nanosci. Nanotechnol.* 11 (2011) 661–665. doi:10.1166/jnn.2011.3194.
- [48] K. Prashantha, M.F. Lacrampe, P. Krawczak, Highly dispersed polyamide-11/halloysite nanocomposites: Thermal, rheological, optical, dielectric, and mechanical properties, *J. Appl. Polym. Sci.* 130 (2013) 313–321. doi:10.1002/app.39160.
- [49] J.W. Sikora, I. Gajdoš, A. Puszka, Polyethylene-Matrix Composites with Halloysite Nanotubes with Enhanced Physical/Thermal Properties, *Polymers (Basel)*. 11 (2019) 787. doi:10.3390/polym11050787.
- [50] M. Liu, Y. Shen, P. Ao, L. Dai, Z. Liu, C. Zhou, The improvement of hemostatic and wound healing property of chitosan by halloysite nanotubes, *RSC Adv.* 4 (2014) 23540–23553. doi:10.1039/C4RA02189D.
- [51] G. Biddeci, G. Cavallaro, F. Di Blasi, G. Lazzara, M. Massaro, S. Milioto, F. Parisi, S. Riela, G. Spinelli, Halloysite nanotubes loaded with peppermint essential oil as filler for functional biopolymer film, *Carbohydr. Polym.* 152 (2016) 548–557. doi:10.1016/j.carbpol.2016.07.041.
- [52] Y. Chen, L.M. Geever, J.A. Killion, J.G. Lyons, C.L. Higginbotham, D.M. Devine, Halloysite nanotube reinforced polylactic acid composite, *Polym. Compos.* 38 (2017) 2166–2173. doi:10.1002/pc.23794.

CHAPTER 1

Halloysite nanotubes functionalization with phosphonic acids: role of surface charge on molecule localization and adsorption reversibility

As described in the previous section, the HNT fairly unique structural ambivalence is a powerful tool that might enable to modify separately the inner and outer surfaces, assigning them different tasks: in nanomedicine, theranostic applications could be envisioned, coupling transport of active molecules[1–4] and diagnostics via triggered luminescent probes[5]; in nanocomposites and functional coatings, the outer surface of HNT could be modified to enhance compatibility with the matrix, while the inner lumen could be loaded with functional compounds (eliciting *e.g.* anti-microbial or self-healing properties).

Many literature studies about the selective modification of HNT surfaces report the loading of active principles on halloysite through electrostatic interactions. Some examples include the loading of ibuprofen[6], or diclofenac sodium molecules[7], as well as chitosan-modified HNT loaded with aspirin[8]. While electrostatic loading can be easier to perform, as often it only requires vacuum cycles and a controlled solution pH to load the active principle selectively into the inner lumen, it presents some drawbacks: electrostatic forces are generally not selective enough, causing a fast and uncontrolled release of the biomolecule, which is likely to occur far away from the desired target in the organism[9]. This poses also some concerns about the storage of such nanosystems, as active principles could diffuse in the storing solution, *de facto* decreasing the potency of the therapeutic agent and drastically lowering their shelf-life.

On the other hand, the covalent modification of halloysite surface, while usually more difficult, allows for better control over the release of loaded compounds, which can be tuned to happen only under specific conditions, *i.e.* lysosomal pH or enzymatic activity. This greatly increases the potency and targeting ability of covalently-modified HNT systems, making them valuable tools for the battle against insidious pathologies as cancer. Covalent grafting also prolongs the shelf life of nanocarriers, simplifying their storage and keeping their efficacy unaltered.

Nonetheless, selective functionalization of halloysite nanotubes via covalent linkers is scarcely investigated in the literature, as precisely defining the nature and location of molecule adsorption is a complex matter.

To help clarify this issue, in this work surface modification of halloysite was carried out together with the functionalization of purposely prepared model oxides mimicking the inner and outer surfaces.

To this task, phosphonic acids were chosen as functionalizing agents: they are hetero-organic compounds bearing a C-PO(OH)_2 group, which are known for adsorbing covalently on oxide substrates and can be used to generate self-assembled monolayers (Figure 1.1A)[10,11]. Contrary to organosilanes, they do not suffer from homocondensation and their selectivity towards certain oxides[12] can be exploited for the selective functionalization of inherently dual systems, such as halloysite nanotubes. Although the literature reports several examples illustrating phosphonates inability to graft onto SiO_2 substrates in aqueous conditions[13,14], little is known about the real mechanism behind this behaviour. Various reports simply ascribe it to a generic susceptibility of Si-O-P bonds to hydrolysis[15–19], while some suggest that in humid conditions a network of hydroxyls renders the SiO_2 surface unreactive[20].

Although a few literature studies report the modification of halloysite nanotubes with phosphonates[21–23], most of them simply use phosphonic acids as a surface modification strategy[21,22] without investigating the nature of the bond or its location. To date, the seminal paper by Yah et al.[24] remains the only paper investigating the location and adsorption mode of alkylphosphonic acids on halloysite nanotubes. However, the role of surface charge on the adsorption mechanism and the reversibility of the bond were not investigated in that paper and, to the author's best knowledge, no previous reports on these topics can be found in the literature. For this reason, in the present work, particular attention was devoted to understanding the adsorption nature and gauging its reversibility, focusing on the role of the substrate surface charge. To this end, model oxides, silica and alumina (oxo)hydroxide powders, were also adopted to mimick the outer and inner surfaces of HNT, respectively.

Octylphosphonic acid (OPA), shown in Figure 1.1B, was selected as functionalizing agent since its alkyl chain made it possible to monitor surface modification both by FTIR spectroscopy and macroscopical changes in surface properties, such as wetting and dispersibility. The role of different adsorption parameters was investigated, such as OPA concentration, reaction time and solution pH, while also checking adsorption reversibility.

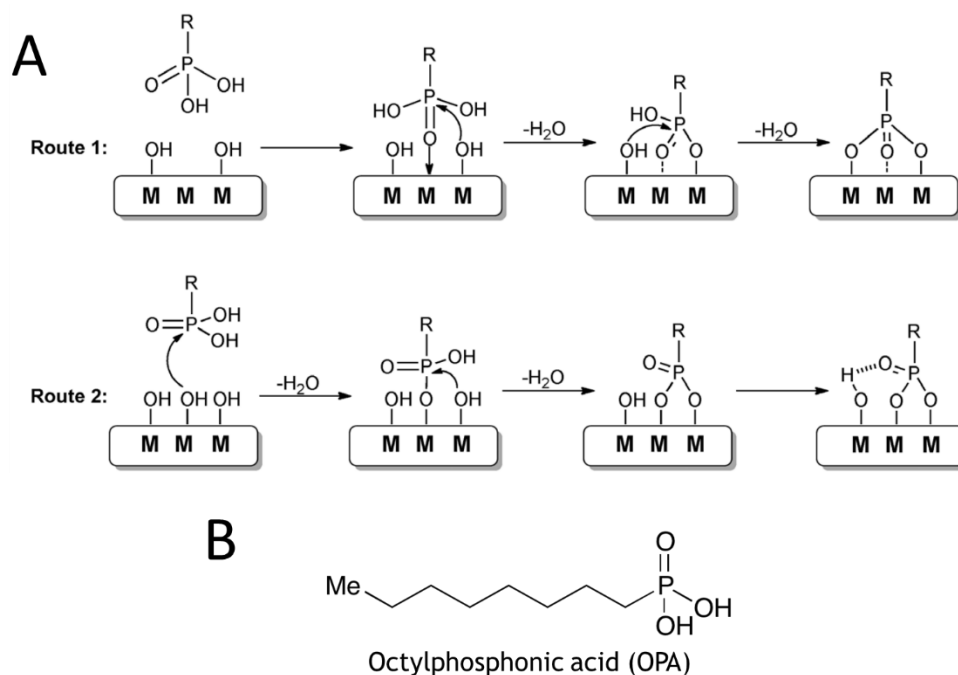


Figure 1.1 - Mechanism of phosphonic acid attachment to Lewis acidic metal oxides (route 1) and poorly Lewis acidic metal oxides (route 2) (A; Zuilhof et al., *Angew. Chem. Int. Ed.*, 53, 2014) and chemical structure of octylphosphonic acid (B).

1.1 Experimental procedures

1.1.1 Materials

Halloysite nanotubes and chemicals were acquired from Sigma-Aldrich (unless differently stated) and were used without further purification; water was doubly distilled and deionised through a Milli-Q apparatus.

1.1.2 Preparation of model oxides

The adsorption of phosphonic acids at the surface of model oxides was investigated using silica and aluminium (oxo)hydroxide as models of the outer and inner surfaces of HNT, respectively. In order to ensure the general validity of the results, both in-house synthesized and commercial oxides (SiO_2 from Sigma-Aldrich, $\text{AlO}(\text{OH})$ from Sasol) were adopted; the latter samples are identified in the text with a “c_” prefix. In order to better understand the effect of surface charge on the functionalization mechanism, in-house synthesized TiO_2 particles were also used for comparison in surface charge studies. Owing to the TiO_2 intermediate isoelectric point with respect to the other investigated oxides, the effect of a positive or negative surface charge on the adsorption mechanism could be evaluated by simply changing the functionalization pH in the 4-9 range, while keeping constant the oxide nature and morphology.

SiO_2 particles were prepared via a modified Stöber synthesis[25] using the following procedure: 5 mL of tetraethyl orthosilicate (TEOS), 75 mL of 99.8% ethanol and 10 mL

of ammonium hydroxide (NH₄OH, 25%) were mixed, agitated for 30 s and then kept at room temperature for 4 h. The condensed phase was retrieved and washed nine times by centrifugation-resuspension cycles with distilled water, reaching neutral pH, after which it was dried at 70 °C for 24 h.

Al(OH)₃ particles were synthesized by forced hydrolysis, adapting the procedure reported by Kang et al.[26]. Al(NO₃)₃ · 9 H₂O (2.2 mmol) and Al₂(SO₄)₃ · 16 H₂O (0.32 mmol) were added to 280 mL of 0.1 M urea aqueous solution. The solution was kept at 98 °C without stirring for 4 h, during which a white solid started flocculating. Finally, the suspension was cooled to stop the reaction and the condensed phase was collected. The product was washed five times with water via centrifugation-resuspension cycles and dried at 100 °C for 24 h.

Finally, TiO₂ nanoparticles were prepared by a sol–gel synthesis according to a previously reported procedure[27]. Briefly, a solution of Ti(OC₃H₇)₄ in 2-propanol was hydrolysed at 65 °C by addition of water, adopting a water/alkoxide molar ratio of 100 and a water/2-propanol molar ratio of 20. The obtained precipitate was dried overnight at 90 °C, and then thermally treated at 300 °C for 5 h under O₂ stream.

1.1.3 Optimization of surface functionalization conditions

Surface functionalization of model oxides was conducted as follows. The oxide powder (200 mg) was suspended in 16 mL of a 1:1 water/2-propanol mixture and conditioned via sonication for 5 min. After the addition of the required amount of OPA, the suspension was stirred at room temperature (unless differently stated) for 1 h. For selected samples, the effect of functionalization temperature on adsorption was investigated in the range 0-80 °C. Longer functionalization times (up to 7 h) did not result in any significant improvement in the functionalization degree. The pH was monitored through a pH meter and adjusted at a value of either 4 or 9 with KOH and HNO₃ 0.05 M solutions. Then, the powder was collected via centrifugation and dried at 100 °C for 24 h. Control tests were carried out washing the powder with a hydroalcoholic solution via centrifugation/resuspension cycles, giving fully comparable results. Functionalized samples bear the suffix “–OPA”.

For the sake of comparability among different oxides, nominal OPA amount was considered as surface density δ (*i.e.*, number of OPA molecules per nm²), calculated according to the following equation:

$$\delta \equiv \frac{\text{molecules}_{OPA}}{\text{nm}^2_{sub}} = \frac{m_{OPA}}{MW_{OPA}} \cdot \frac{1}{SSA_{sub} \cdot m_{sub}} \cdot \frac{6.022 \times 10^{23}}{(10^9)^2}$$

where m_{OPA} and m_{sub} are respectively the amounts of OPA and of substrate expressed in g, MW_{OPA} is the molecular weight of OPA (in g mol⁻¹) and SSA_{sub} is the specific surface area of the oxide substrate expressed in m² g⁻¹. OPA surface density for each substrate was varied in the range 0.1-10 molecules nm⁻².

Functionalization of HNT was carried out according to a slightly modified procedure, in order to take into account their tubular morphology. The HNT were purchased from Sigma-Aldrich and used without further purification; their main morphological and

structural characteristics are reported in Table 1.1 and Figure 1.2; nanotubes present a broad size distribution and a noticeable degree of structural defectivity, as their sizes range from 100 nm to 800 nm and most of their edges appear jagged. 250 mg of HNT was added under stirring to a hydroalcoholic solution (4:1 ethanol/water mixture) of OPA (4 mM). The suspension was adjusted to pH 4, then it was transferred to a vacuum jar and evacuated using a vacuum pump. The fizzing of the suspension indicated that air was being removed from the HNT lumen and replaced with the OPA solution. The process of air evacuation and cycling back to atmospheric pressure was repeated three times, according to a procedure reported by Yah et al.[24], in order to maximize OPA loading in the halloysite lumen. After stirring for a week at room temperature, the modified HNT were rinsed and centrifuged five times with a 4:1 ethanol/water mixture; it was then dried at 100 °C under vacuum for 24 h. The final product was labelled HNT-OPA.

1.1.4 Reversibility tests

Tests to assess the reversibility of the OPA adsorption were carried out on oxides previously functionalized at pH 4. Powders were dispersed via sonication in water, adjusting the pH at different values (either pH 4, 7 or 10) using diluted KOH and HNO₃ solutions, and stirred for 1 h. In the case of HNT, three vacuum cycles were also carried out in order to ensure solution access to the inner lumen. Then, the powders were collected by centrifugation and washed with 2-propanol to remove desorbed OPA residues. The recovered powders were then dried in oven at 70 °C.

1.1.5 Sample characterizations

The specific surface area of the samples was determined by means of N₂ adsorption-desorption isotherms in subcritical conditions recorded on a Coulter SA 3100 apparatus and analyzed according to the Brunauer–Emmett-Teller (BET) method. Desorption isotherms were used to determine the pore size distribution using the Barrett, Joyner, and Halenda (BJH) method.

X-ray diffraction (XRD) patterns of the powders were acquired at room temperature using a Siemens D5000 diffractometer equipped with a Cu K α source ($\lambda = 0.15406$ nm), working at 40 kV x 40 mA nominal X-rays power. $\theta : 2\theta$ scans were performed between 10° and 80°, with step size 0.02° wide.

The isoelectric point (pH_{IEP}) was evaluated determining the oxide ζ -potential on a range of pH values using a Zetasizer Nano ZS (Malvern Instruments), operating at $\lambda = 633$ nm with a solid state He-Ne laser at a scattering angle of 173° and using the dip-cell kit. For ζ -potential measurements, powders were suspended in a 0.01 m KNO₃ aqueous solution, while pH was adjusted with diluted KOH and HNO₃ solutions. Average particle size determinations were performed on powder samples dispersed in milliQ water by dynamic light scattering (DLS) using the same instrument. Each hydrodynamic diameter as well as ζ -potential were averaged from at least three measurements.

Transmission electron microscopy (TEM) images were acquired using a Zeiss LEO 912ab Energy Filtering TEM operating at an acceleration voltage of 120 kV, equipped with a CCD-BM/1K system. Samples were dispersed in either 2-propanol or water (1 mg mL⁻¹) and deposited on Cu holey carbon grids (200 mesh).

Fourier transform infra-red (FTIR) spectra were registered using a Perkin Elmer Spectrum 100 spectrophotometer in Attenuated Total Reflectance (ATR) mode using a resolution of 4.0 cm⁻¹ and acquiring 12 scans between 4000 and 400 cm⁻¹.

Dispersibility and suspension stability in water were assessed by measuring the absorbance at 550 nm of a 5 mg mL⁻¹ HNT aqueous suspension at spontaneous pH as a function of time, using a Shimadzu UV2600 spectrophotometer.

Contact angle measurements, θ , were performed on oxide films, prepared by drop casting 1 mg mL⁻¹ suspensions in 2-propanol on glass, using a Krüss EasyDrop instrument. For each film, five static angles were measured, using 2 μ L droplets of water.

Energy-dispersive X-ray spectroscopy (EDX) analyses were carried out using a Hitachi ED3000 probe on a Hitachi TM1000 scanning electron microscope operating at 15 kV.

1.2 Results and discussion

1.2.1 Functionalization of model oxides

The functionalization of aluminium (oxo)hydroxides and silica powders with OPA was investigated as models of the inner and outer surfaces of HNT, respectively. The main characteristics of the adopted model oxides are summarized in Table 1.1. To investigate the role of the oxide nature, deeply different materials, both morphologically and structurally, were chosen. It is noteworthy that comparable results were obtained on both commercial and in-house synthesized samples, supporting a role of the oxide nature irrespective of the specific sample morphological and structural properties. Hence, in the following, only the most relevant samples will be shown.

Surface modification was studied via FTIR spectroscopy and contact angle measurements. Data relative to functionalization carried out at pH 4 will be first presented. FTIR spectroscopy was adopted for the preliminary assessment of the presence of OPA on the oxide surface upon functionalization.

Sample	Phase composition	Average dimension (nm)	SSA (m ² g ⁻¹)	pH _{IEP}
SiO ₂	amorphous	400 ± 90	8	1.9
c_SiO ₂	amorphous	250 ± 80 (80%) 1500 ± 400 (20%)	180	2.3
Al(OH) ₃	gibbsite	160 ± 70	30	9.7
c_AlO(OH)	bohemite	160 ± 20	250	9.0
TiO ₂	anatase 80% brookite 20%	150 ± 70 (80%) 600 ± 100 (20%)	158	4.7
HNT	halloysite, quartz and gibbsite impurities	length: 400 ± 300 outer diameter: 25- 200 inner diameter: 10- 30	57	1.6

Table 1.1 - Morphological and surface properties of bare model oxides: average particle size measured via DLS (hydrodynamic diameter) for the model oxides and via TEM (nanotube length, see Figure 1.2) for HNT; specific surface area (SSA); isoelectric point (pH_{IEP}).

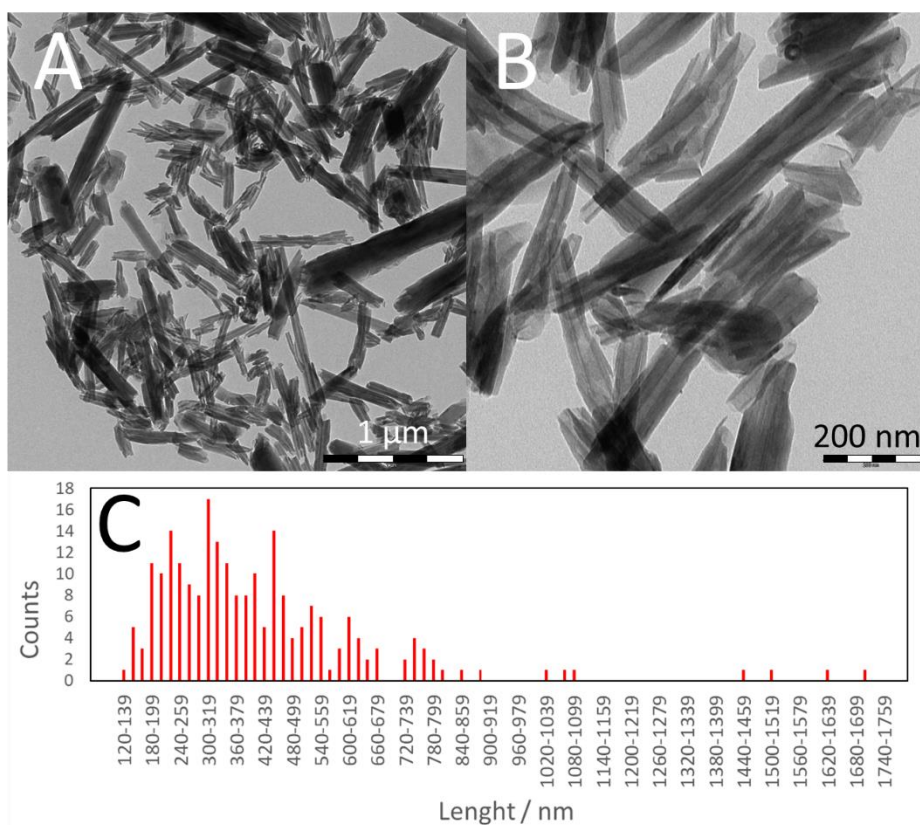


Figure 1.2 - TEM images of halloysite nanotubes (A, B), and size distribution (C).

In Figure 1.3 the FTIR spectra of pristine and OPA-functionalized SiO₂ and Al(OH)₃ are compared. The wavenumber range shown, between 3200 and 2600 cm⁻¹, is typical of

aliphatic CH_x stretching. The related spectra of the TiO₂ reference are reported in Figure 1.4.

After functionalization, both oxides display the characteristic stretching signals of aliphatic chains at 2959, 2925 and 2854 cm⁻¹, belonging to the asymmetrical modes of methyl groups, and the asymmetrical and symmetrical modes of methylene, respectively[28]; the position of the CH_x stretching bands supports a disordered orientation of the alkyl chains[29]. Notwithstanding the difference in specific surface area between the two oxides, the alkyl chains peaks are more appreciable in the case of Al(OH)₃, especially in the spectra of δ₇ and δ₁₀ samples. The TiO₂ spectra show a trend similar to that of Al(OH)₃ samples.

Contact angle measurement is one of the most commonly adopted techniques to assess surface modification[30], as the wetting properties of the support may drastically change after the functionalization. The functionalization effect on the surface wettability was investigated by monitoring the contact angle as a function of the phosphonic acid amount.

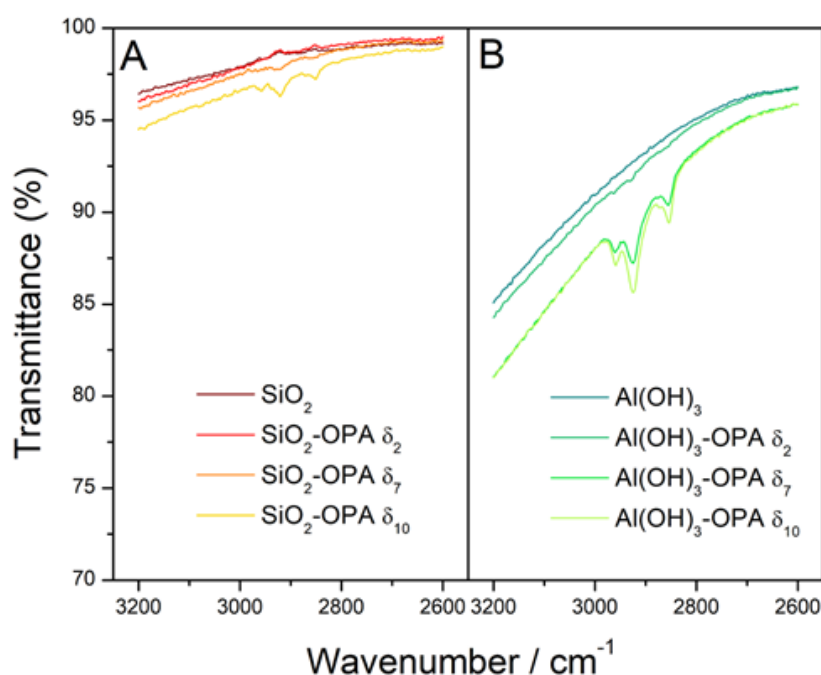


Figure 1.3 - FTIR spectra of pristine and OPA-functionalized SiO₂ (A) and Al(OH)₃ (B); the functionalization was carried out at pH 4.

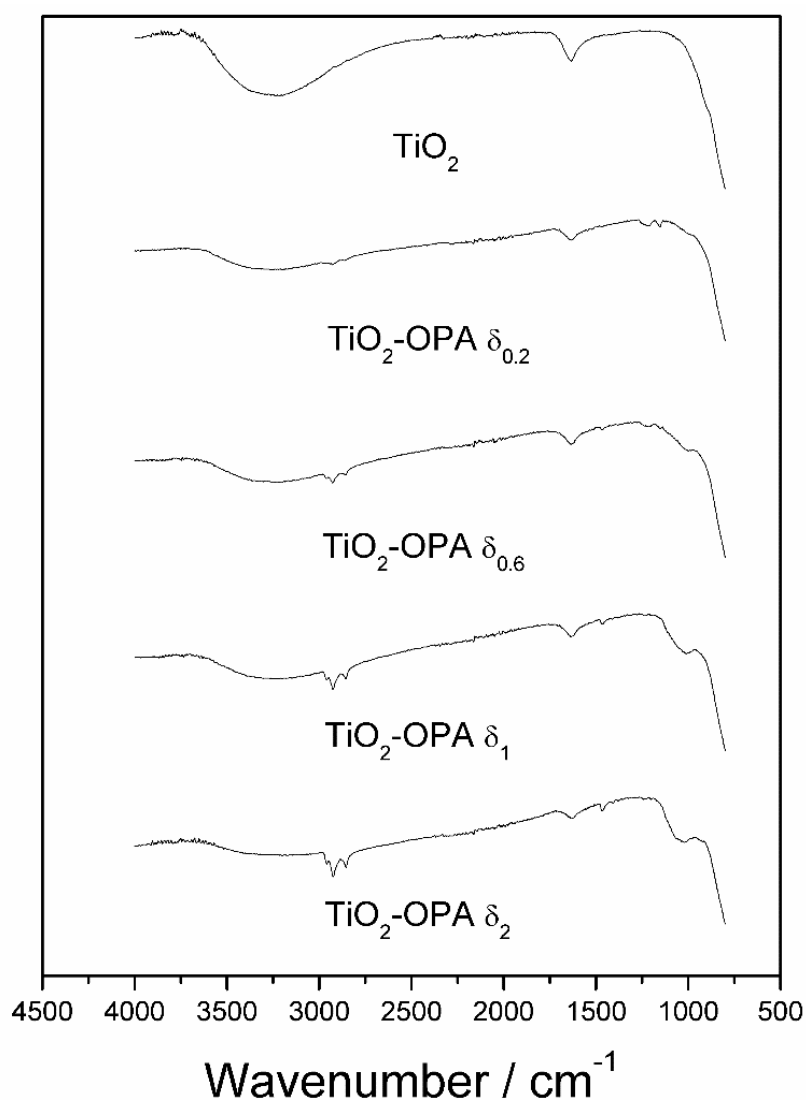


Figure 1.4 - FTIR spectra of bare and functionalized TiO_2 .

In Figure 1.5, the average water contact angles of $\text{Al}(\text{OH})_3$ and SiO_2 films are plotted against the nominal surface density of OPA molecules and compared with those of TiO_2 films as reference. All of the pristine oxides show a complete water spreading. However, upon functionalization, largely different trends are appreciable among the investigated oxides: as the OPA amount increases, the contact angle of the functionalized $\text{Al}(\text{OH})_3$ grows up to around 135° at *ca.* 4 molecules nm^{-2} ; conversely, the wettability of functionalized SiO_2 is hardly affected and sits around 0° . However, when using CH_2I_2 instead of water, the measured contact angles increased with the nominal OPA amount in a rather similar way for each oxide, as can be seen in Figure 1.6. This observation is in agreement with FTIR spectra showing the presence of OPA molecules also on the SiO_2 surface.

These results suggest that OPA molecules on alumina (oxo)hydroxide substrates orient their polar heads towards the surface, exposing the hydrophobic alkyl chains, whereas on silica the phosphonic head forms labile interactions with the oxide and as a result, molecules are solubilised by the probe polar solvent, *i.e.* water.

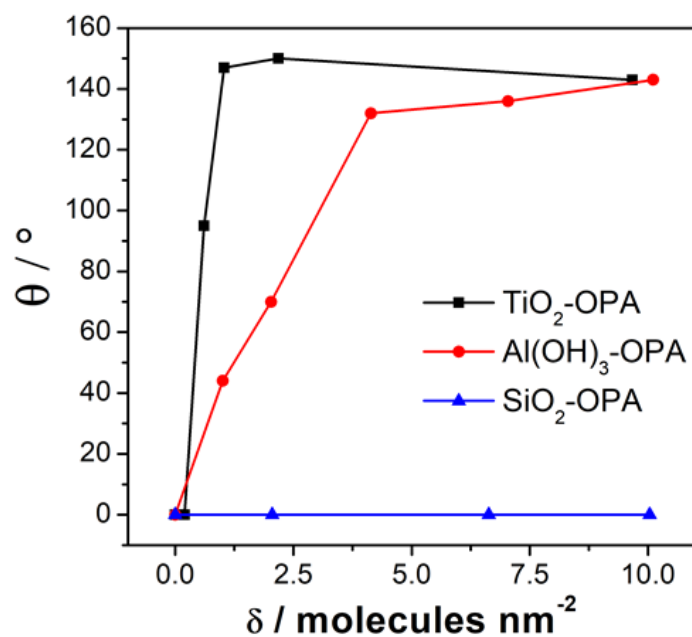


Figure 1.5 – Water contact angles of functionalized oxide films as a function of the nominal OPA surface coverage (functionalization at pH 4). Error bars are in the order of the markers size.

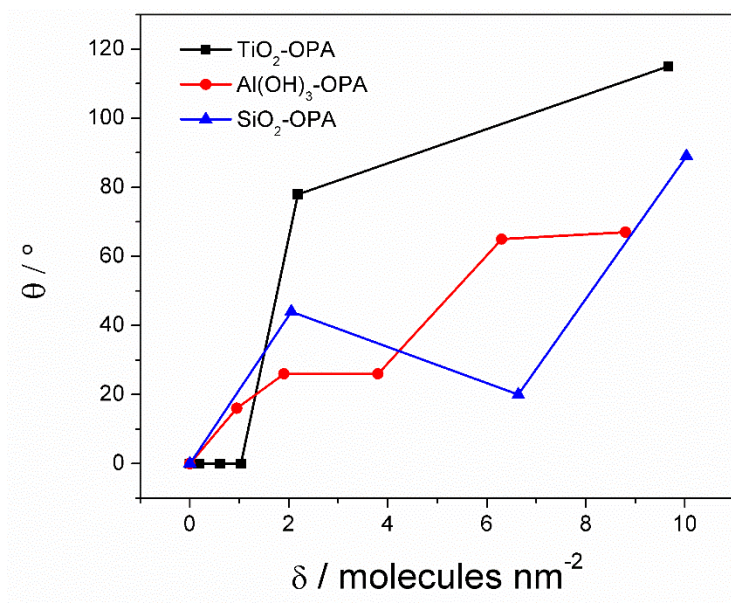


Figure 1.6 - CH_2I_2 contact angle of the pristine and functionalized oxides as a function of the OPA content.

Figure 1.7 shows the differential FTIR spectra for the three investigated oxides in the region between 1500 and 800 cm^{-1} , where the phosphonate head signals can be found. All oxides present a band at *ca.* 1460 cm^{-1} which can be attributed to the scissoring mode of CH_2 [31]. Functionalized SiO_2 samples show no signal but those relative to free

phosphonate P-O stretching and P-O-H bending at 1000 and 950 cm^{-1} , respectively[32], confirming the lack of Si-O-P bonds which should be instead appreciable at ca. 1080 cm^{-1} [33]. Aluminium hydroxide spectra show no free P=O stretching band at 1220 cm^{-1} , indicative of unbound OPA molecules, although a slight signal at 1220 cm^{-1} becomes appreciable only when a surface coverage plateau is reached (deduced from contact angle measurements). Moreover, the $\text{Al}(\text{OH})_3$ spectra present a broad band centred at 1050 cm^{-1} , which could be the convolution of several components. In this respect, the $\text{AlO}(\text{OH})$ spectra (Figure 1.8) show sharper peaks in this region: a main component at 1070 cm^{-1} , a second peak at 1150 cm^{-1} and a shoulder at 1000 cm^{-1} , which can be attributed to stretching modes of Al-O-P, bound P=O and P-O, respectively. It should be noted that these three components show an appreciable shift to lower frequencies for increasing OPA surface density. On the other hand, TiO_2 shows a different behaviour: at lower surface densities, both free and bound P=O signals can be appreciated at 1220 and 1150 cm^{-1} respectively, together with the bands at 1070 and 1000 cm^{-1} , relative to Ti-O-P moieties and free P-O[34]. When surface coverage increases, the peak at 1220 cm^{-1} disappears, while the three components at 1150, 1070 and 1000 cm^{-1} merge into a single broad band.

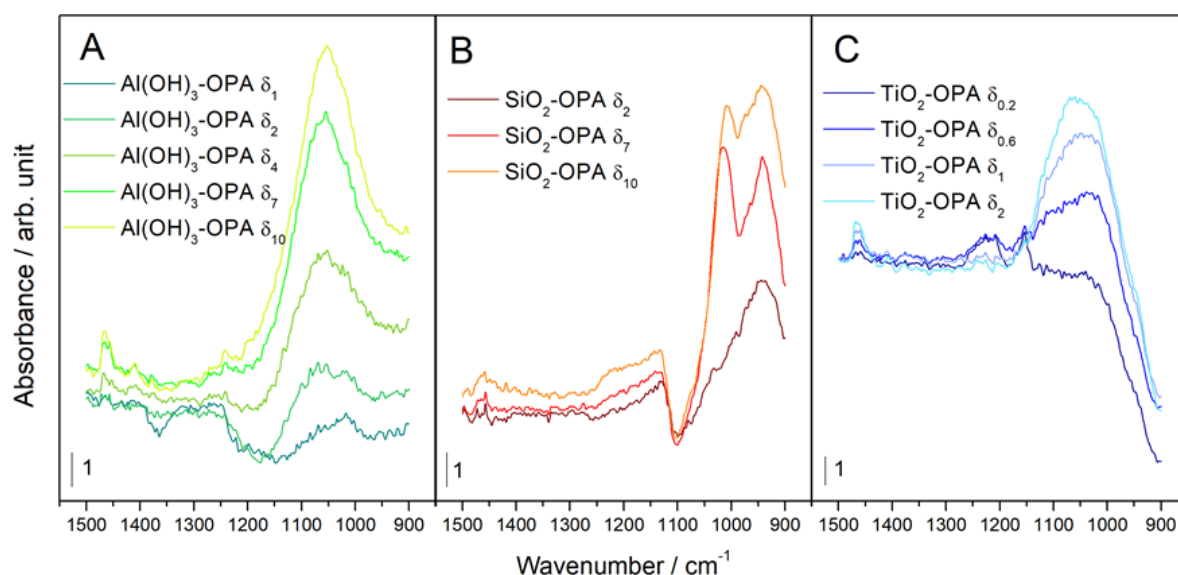


Figure 1.7 – Differential FTIR spectra of functionalized $\text{Al}(\text{OH})_3$ (A), SiO_2 (B) and TiO_2 (C), obtained by subtracting the relative spectrum of the pristine oxide; functionalization was carried out at pH 4.

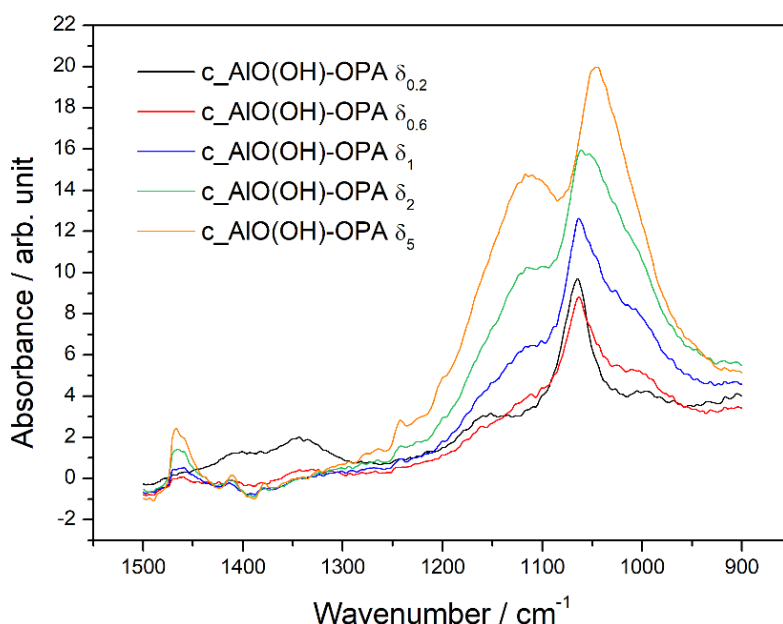


Figure 1.8 - Differential FTIR spectra of OPA-functionalized commercial AlO(OH), referenced to the pristine oxide.

These observations can be explained on the grounds of the different Lewis acidity of aluminium (oxo)hydroxide and TiO₂, which affects OPA coordination: in the case of Lewis acidic metal oxides such as aluminium (oxo)hydroxide, OPA will adsorb on the surface coordinating its P=O moiety first and then will form Al-O-P bonds with its P-OH groups. Conversely, in the case of poor Lewis acids, nucleophilic M-OH groups attack the P centre, leading then to surface binding with a minor contribution of P=O[32,35]. TiO₂ being a poorer Lewis acid compared to aluminium (oxo)hydroxides, the OPA molecule coordination appears to fall in between the two descriptions, as supported by the presence of both types of P=O signals.

The effect of temperature on adsorption was tested in the 0-80°C range. Some differences are appreciable both in terms of contact angle and FTIR spectra (Figure 1.9). Samples prepared at 25°C show slightly higher contact angles than samples prepared at 0 °C and 80 °C (Figure 1.9A) and a more intense band at 1070 cm⁻¹ in FTIR spectra, which is associated with the Al-O-P bond (Figure 1.9B), suggesting that the best temperature to promote the binding of OPA, in the present conditions, is 25°C.

To better understand the nature of adsorbent-adsorbate interaction, the role of the pH of functionalization was investigated with respect to the surface charge of each oxide and of the speciation of the functionalizing agent. To this aim, functionalization tests were conducted at either pH 4 or 9. The relative contact angle isotherms are reported in Figure 1.10.

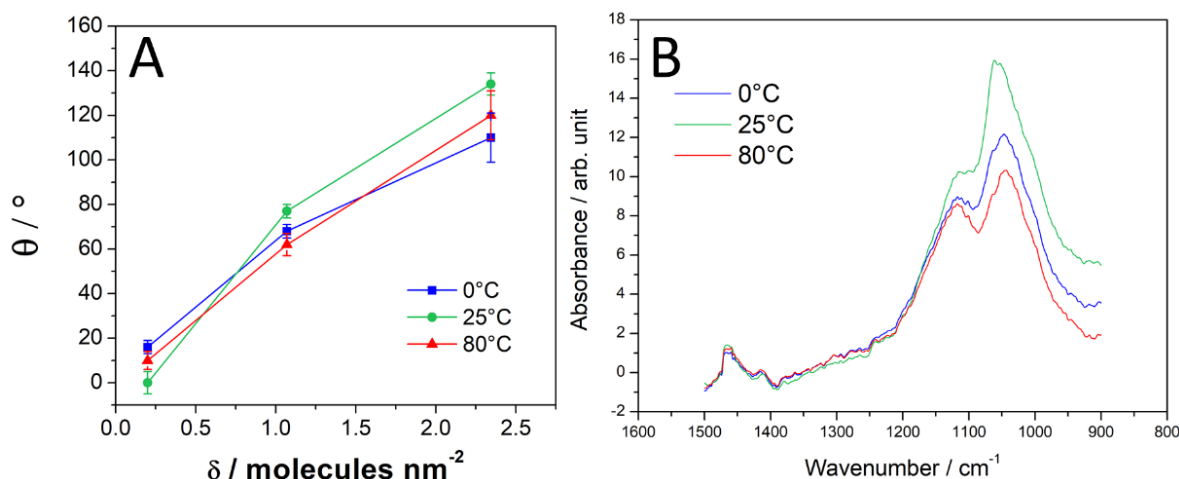


Figure 1.9 - Water contact angle isotherms (A) and differential FTIR spectra (B) of commercial AlO(OH) functionalized with OPA at different temperatures. The FTIR spectra are relative to samples with a δ of 2.3.

While for both SiO_2 (not shown) and Al(OH)_3 increasing the functionalization pH has a negligible effect, in the case of TiO_2 the impact was visible, especially for lower OPA amounts, and hydrophobic plateau could not be reached even at higher OPA densities. The observed difference in behaviour between oxides can be rationalized on the grounds of their surface charge properties, in particular of their pH_{IEP} values. Figure 1.11 reports the ζ -potential curves used for isoelectric point determinations. The measured pH_{IEP} values, reported in the inset table, are in good agreement with literature data[36].

According to pH_{IEP} values, SiO_2 is negatively charged at both the investigated functionalization pH values, whereas Al(OH)_3 is positively charged at both pH values. Conversely, the surface charge of TiO_2 was positively charged at pH 4 and negatively charged at pH 9. Considering that the pK_{a1} and pK_{a2} of OPA are respectively at 2.7 and 8.2[37], the OPA molecule is negatively charged due to deprotonation at both the investigated pH values, more so at pH 9. We can thus conclude that electrostatic repulsion played a fundamental role in determining the susceptibility to hydrolysis of the M-O-P bond, which was enhanced by negative surface charge, as it will be more extensively discussed in Section 3.

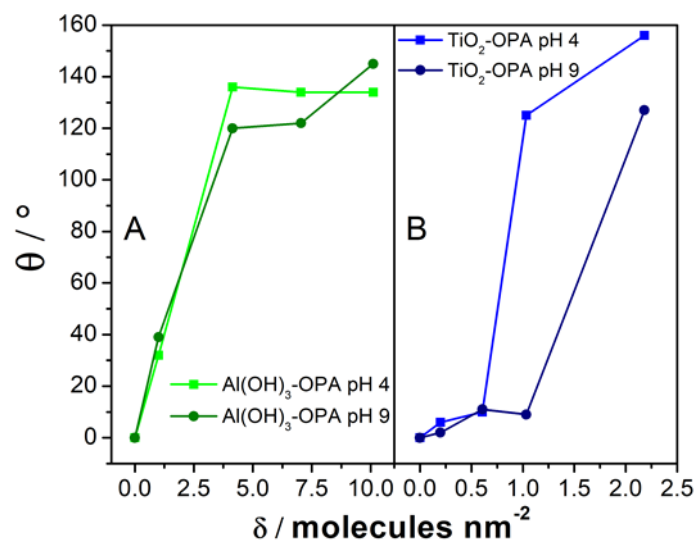


Figure 1.10 – Water average contact angle vs. OPA nominal surface density for Al(OH)_3 (A) and TiO_2 (B) impregnated either at pH 4 or 9.

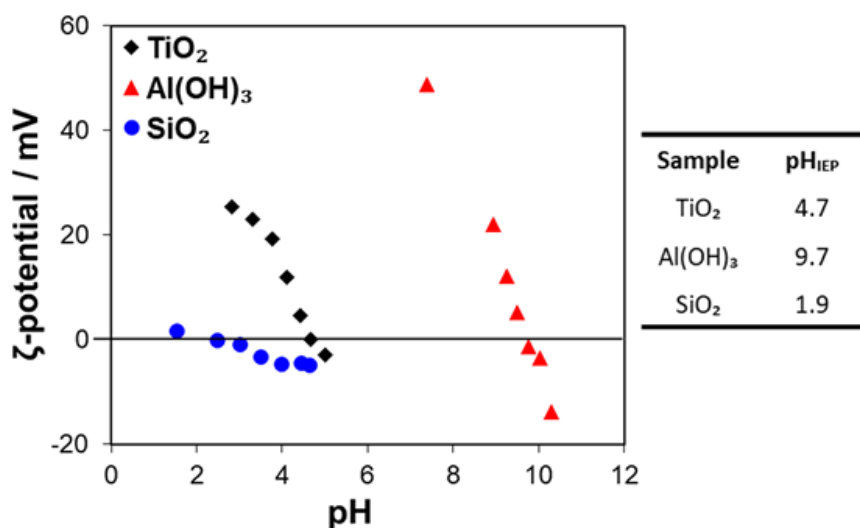


Figure 1.11 – ζ -potential curves as a function of pH and the resulting pH_{IEP} values (inset) for pristine TiO_2 , Al(OH)_3 and SiO_2 .

1.2.2 Characterization of HNT-OPA

Figure 1.12 reports the FTIR spectra of HNT before and after functionalization with OPA, in the frequency region between 2800 and 3800 cm^{-1} , characteristic of OH and CH_x stretching signals, where the main changes are appreciable.

The untreated HNT sample shows three main signals: peaks at 3692 and 3622 cm^{-1} can be attributed to the stretching of inner and interlayer $\text{Al}-(\mu_2\text{-OH})\text{-Al}$ bridges, respectively[38], while the band at 3548 cm^{-1} can be assigned to the stretching of the hydrogen bonded OH groups of intercalated water[39]. Beneath these sharper peaks, the

broad band of O-H stretching, due to physisorbed water, extends from 3750 cm^{-1} to 3000 cm^{-1} . After the functionalization, a general intensity decrease is observed in the O-H stretching region: this might be related to a loss of surface hydration[40]. Moreover, the appearance of the characteristic CH_x stretching bands at 2956 , 2926 and 2853 cm^{-1} , confirmed the presence of disordered OPA aliphatic chains[28]. Figure 1.13 compares the FTIR spectra of HNT and HNT-OPA in the $1500\text{-}800\text{ cm}^{-1}$ region, along with their differential spectrum. Beside the CH_2 deformation band at 1460 cm^{-1} , other signals attributable to OPA are present. The two bands at 1203 and 1156 cm^{-1} might be due to the stretching of free and bound P=O groups, while the sharp peak at 1083 cm^{-1} could be attributed to Al-O-P stretching. A small peak at 985 cm^{-1} is noticeable. The occurrence of both free and bound P=O stretching supports the ^{31}P solid state NMR findings reported by Yah and co-authors[24] suggesting the copresence of bi- and tridentate binding modes. Furthermore, the presence of the two different P=O components might suggest that the OPA-HNT mode of adsorption does not closely mirror the OPA-Al (oxo)hydroxide interactions shown in Figure 1.7A and Figure 1.8. In this respect, the use of models allowed to shine light onto the inherently complex P-O stretching region.

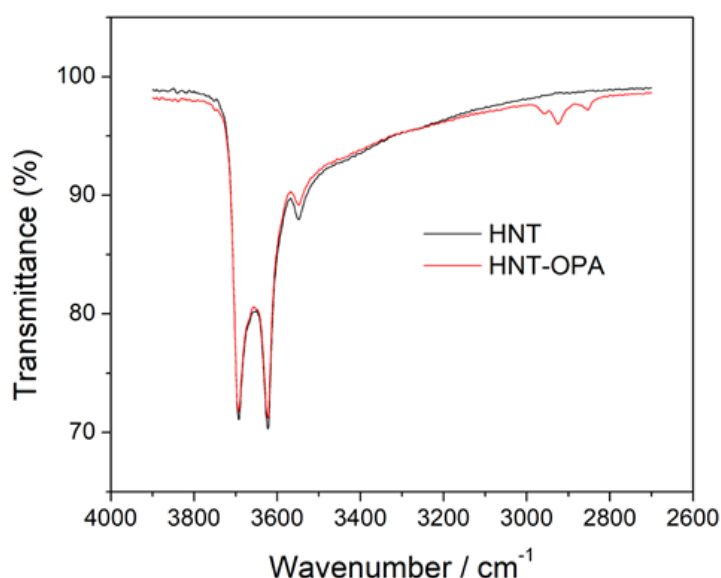


Figure 1.12 – FTIR spectra of pristine and OPA-functionalized HNT in the $4000\text{-}2600\text{ cm}^{-1}$ region.

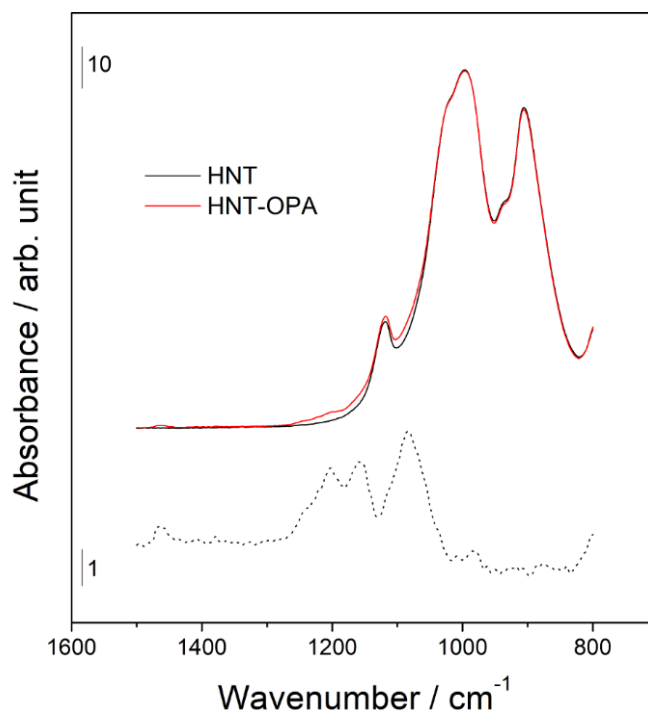


Figure 1.13 - FTIR spectra of HNT and HNT-OPA in the 1500-800 cm^{-1} region. The dotted line is the differential spectrum.

N_2 adsorption-desorption isotherms in subcritical conditions carried out on bare HNT yielded a specific surface area of $53 \text{ m}^2 \text{ g}^{-1}$, in line with previously reported values[41]. After functionalization, the sample showed a 13% decrease in surface area ($46 \text{ m}^2 \text{ g}^{-1}$). Changes in porosity were also observed: while the total pore volume did not decrease by a great margin (from 0.338 to 0.307 mL g^{-1} , about 9%), a notable loss of micropores could be observed (Figure 1.14A). Similar results have been reported for the surface functionalization of oxides with organosilanes[27,42]. In the present case, the OPA molecules (*ca.* 1 nm in length) might clog the smaller pores. The small peak in the pore size distribution of the pristine material at around 20 nm can be related to the inner lumen of the nanotubes, as shown by TEM images (Figure 1.14B and Figure 1.2) reporting an inner lumen of 10-30 nm. By comparing the pore size distributions before and after OPA functionalization, it is clear that this peak is not affected by the presence of OPA, suggesting that the lumen is not occluded by organic matter. Conversely, Yah et al.[24] reported a much higher loss in specific surface area and a decrease in pore size relative to pores of *ca.* 25 nm; these differences can be explained by the much longer alkyl chain (18 C atoms against 8 C) of the phosphonic acid derivative used by Yah and co-authors, which hindered the inner lumen accessibility.

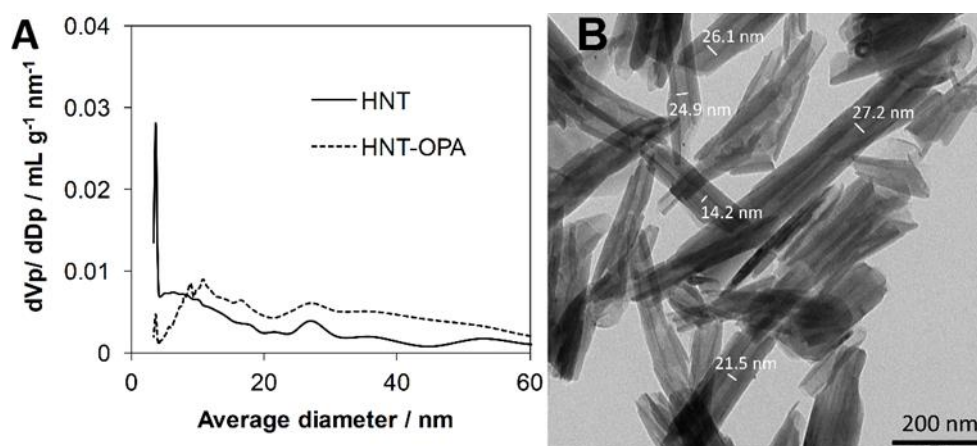


Figure 1.14 – Pore size distribution of HNT before and after functionalization (A); TEM image of pristine HNT (B).

Figure 1.15 reports ζ -potential measurements of the pristine and functionalized HNT as a function of pH. The ζ -potential curves of HNT and HNT-OPA differ significantly for pH values higher than 2.5, the latter sample being more negatively charged. The two curves converge at lower pH values, resulting in the same pH_{IEP} of around 1.5, a typical value for HNT[43]. The difference between the two curves can be traced back to the presence of OPA on the inner surface of functionalized HNT: the covalent binding of OPA decreases the amount of free Al-OH groups, which are responsible for the positive charge of the inner surface, similarly to what previously reported in the case of anionic surfactants[44]. Furthermore, above its pK_{a1} at 2.8, OPA is deprotonated and its negative charge sums up with the basal negative charge of HNT[45]. For pH values more acidic than 2.8, the OPA negative charge is neutralized by protonation and stops affecting the ζ -potential of HNT.

Water dispersibility and dispersion stability of both pristine and functionalized HNT were compared at spontaneous pH (*ca.* 4). Both samples presented fully comparable reflectance at the selected wavelength (550 nm), hence differences in absorbance can be related to the actual dispersion concentration. Initial absorbance was 0.32 and 0.45 for HNT and HNT-OPA, respectively. Figure 1.15B reports the samples normalized absorbance over time to compare the suspension stability. The functionalized HNT displayed a better dispersibility and suspension stability than the pristine sample. It should be noted that the model oxides functionalized with OPA present a much lower dispersibility in water than the bare analogue (particles float in aqueous solvent, Figure 1.16) due to the hydrophobic properties imparted by the OPA coating. The higher dispersibility of HNT-OPA suggests that no OPA was bound on the external SiO_2 surface, which thus retains its hydrophilic properties. The enhanced suspension stability can possibly be explained on the grounds of the higher negative charge of the functionalized sample, as reported by ζ -potential measurements, which may have contributed to increasing electrostatic repulsion between particles, enhancing their stability. Overall, our results are in good agreement with what reported in the literature: in their work, Yah et al. performed XPS analyses on halloysite loaded with alkyl phosphonic acids[24]. Upon functionalization, no alteration of the Si peak of HNT was

recorded, suggesting that the phosphonate groups did not react with surface Si-OH moieties.

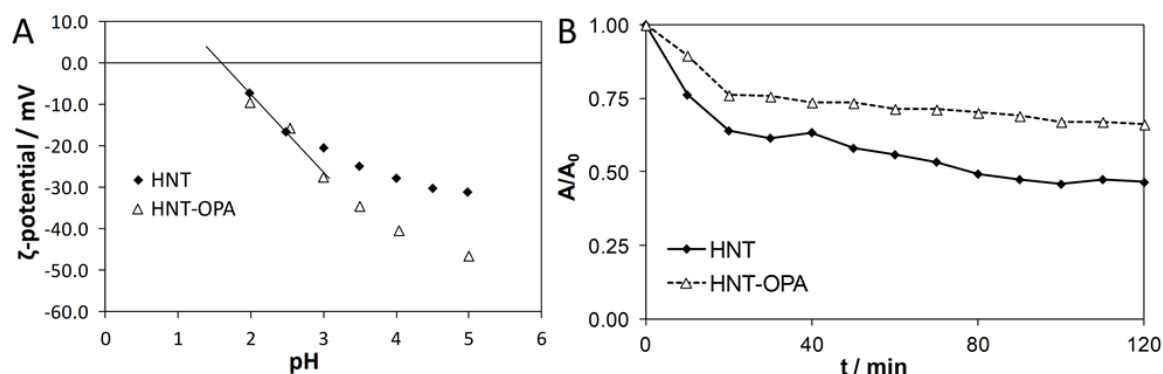


Figure 1.15 – ζ -potential curves before and after functionalization (A) and normalized absorbance (at 550 nm) of suspensions of pristine and OPA-functionalized HNT in water over time (B).

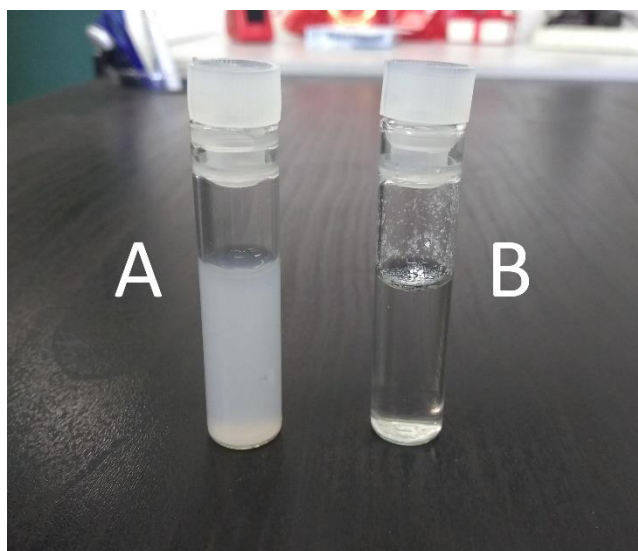


Figure 1.16 - Water dispersion of pristine Al(OH)₃ (A) and Al(OH)₃-OPA δ_4 (B) after sonication for 5 min.

1.2.3 Reversibility of OPA adsorption

The reversibility of OPA adsorption was investigated for both the model oxides and HNT, upon treatment in a range of pH values. To the author's best knowledge, the reversibility of the bond of alkyl chain phosphonic acids on halloysite nanotubes has not been previously investigated in the literature. In this respect, it should be noted that, in the work by Yah and co-workers[24], release tests of ferrocene derivatives did not consider the reversibility of the phosphonic acid bond with the oxide, as the ferrocene

compounds were merely physisorbed due to compatibility with the hydrophobic alkyl chains. Due to the previously discussed labile nature of the OPA adsorption on silica, results relative to SiO_2 samples are not presented in this section.

Figure 1.17 shows the effect of a treatment at pH 10 on the wetting properties of various oxides. While all samples exhibited quite comparable wetting features (contact angles $> 130^\circ$) upon OPA functionalization, notable differences are appreciable among the different oxides after treatment in alkaline environment. TiO_2 shows a complete water spreading upon treatment in alkaline conditions, which is indicative of a loss of OPA coverage. Aluminium (oxo)hydroxides show instead a more complex behaviour: $\text{Al}(\text{OH})_3$ retains the surface functionalization upon treatment at pH 10, as shown by the water contact angle (Figure 1.17A,B) whereas $\text{c_AlO}(\text{OH})$ shows a marked loss in hydrophobicity upon treatment (Figure 1.17C,D), even though the surface does not recover the superhydrophilic properties of the pristine sample. These observations can be explained on the grounds of the pH_{IEP} of the substrate: TiO_2 suffers a drastic loss of OPA upon treatment at a pH value much higher than its isoelectric point (Figure 1.11 inset), whereas in the case of $\text{Al}(\text{OH})_3$, a treatment at the same pH value has hardly any effect thanks to the sample very alkaline pH_{IEP} (close to 10). Notably, $\text{c_AlO}(\text{OH})$ shows an intermediate behaviour in terms of OPA release, due to its lower pH_{IEP} (9.0).

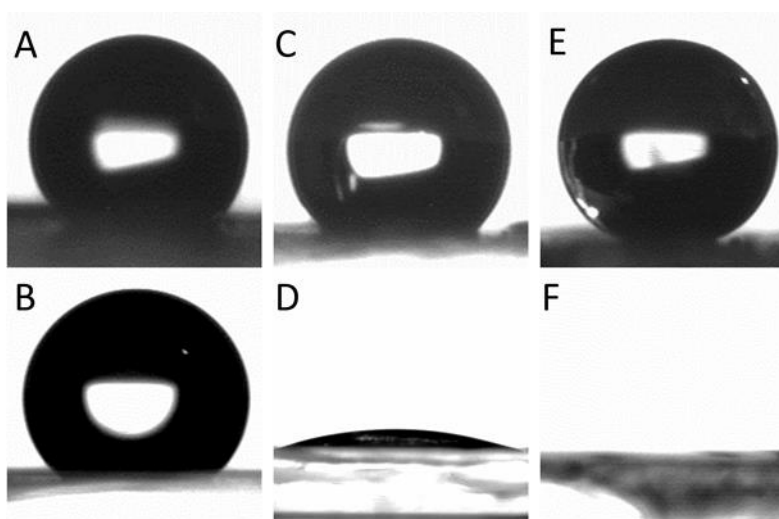


Figure 1.17 – Water contact angles on OPA-functionalized $\text{Al}(\text{OH})_3$ (A, B), $\text{c_AlO}(\text{OH})$ (C, D) and TiO_2 (E, F) before and after treatment at pH 10.

P/(Al,Ti) molar ratio / %				
Sample	Functionalized	After reversibility test		
		pH 4	pH 7	pH 10
Al(OH) ₃ -OPA δ ₁₀	6.6 ± 0.6	7.8 ± 0.6	7.7 ± 0.7	6.4 ± 0.7
c_AlO(OH)-OPA δ ₂	10.5 ± 0.3	10.2 ± 0.3	7.9 ± 0.8	5 ± 1
TiO ₂ -OPA δ ₂	1.8 ± 0.2	1.4 ± 0.2	1.2 ± 0.1	< DL
HNT-OPA	6 ± 1	7.3 ± 0.9	< DL	< DL

Water contact angle / °				
Sample	Functionalized	After reversibility test		
		pH 4	pH 7	pH 10
Al(OH) ₃ -OPA δ ₁₀	149 ± 1	151 ± 2	152 ± 2	138 ± 3
c_AlO(OH)-OPA δ ₂	134 ± 5	122 ± 3	120 ± 19	17 ± 2
TiO ₂ -OPA δ ₂	156 ± 4	148 ± 7	85 ± 22	< 5

Table 1.2 – Phosphorus content (from EDX) and water contact angle values of OPA functionalized samples before and after treatment at various pH values. DL stands for detection limit.

Table 1.2 reports water contact angles and P/metal ratios, determined by EDX, for functionalized samples before and after reversibility tests at different pH values (4, 7 or 10). EDX results compare well with the calculated OPA surface density, within the experimental limits of the technique. Surface wetting and the phosphorus content show comparable trends, confirming that the decrease in hydrophobicity is related to the loss of adsorbed OPA molecules. Results confirm the hypothesized trend based on the surface charge properties of each oxide: while treatment at pH 4 showed negligible

effects in terms of both surface wetting and phosphorus amount for all the investigated oxides, TiO_2 and aluminium (oxo)hydroxides powders treated at pH values higher than their pH_{IEP} suffered a loss of OPA coverage. Interestingly, OPA loss is not complete upon treatment at pH values close or slightly higher than the pH_{IEP} , as shown by tests with TiO_2 at pH 7 and $\text{c_AlO}(\text{OH})$ at pH 10, which indicates that the OPA-oxide interaction is not merely based on electrostatic interactions, in agreement with FTIR results showing the occurrence of M-O-P bonds. Nonetheless, our results suggest that the initial driving force for molecule grafting is mainly electrostatic, and that surface charge plays a role also on the stability of the bond.

The case of HNT-OPA is more complex given that the measured isoelectric point is a global parameter that does not account for the different protonation equilibria of the two surfaces[43]. Despite the very acidic pH_{IEP} of HNT, treatment at pH 4 does not significantly reduce the OPA adsorption, whereas a drastic decrease in OPA content is appreciable for treatment in neutral and alkaline conditions. As reported by Bretti et al.[9,46], at pH 4 about half of the outer Si-OH sites and all of the inner Al-OH ones are protonated. When increasing the pH to 7 the whole SiO_2 surface is deprotonated, together with part of the inner surface. At pH 10 both surfaces are almost completely deprotonated. The present results can be rationalized considering that at neutral pH the effective HNT surface charge is negative enough to detach adsorbed OPA.

1.3 Conclusions

An in-depth understanding of molecule adsorption onto structurally complex materials, such as halloysite, is essential whenever fine tuning of surface features is sought after. Functionalization with phosphonic acids can provide a selective functionalization of the inner lumen of HNT, as shown in the present study by model oxides and complementary characterizations. Upon functionalization, HNT showed enhanced dispersibility in water as a result of the increased surface charge, proving this surface modification compatible with usage in aqueous conditions. Moreover, accessibility to the inner lumen, a key parameter for further loading of compounds, was retained. The reversibility of the OPA-HNT bond was investigated, showing a pH sensitive behaviour, dependent on the oxide surface charge. In this respect, oxide models proved crucial in understanding the relation between support and adsorbate, showing a key role of surface charge on the bond formation and stability. Three model oxides bearing different surface charge features were purposely chosen to compare their behaviour. For example, tests performed on TiO_2 at pH 4 and 9 show a clear difference in adsorption and reversibility, due to the oxide opposite surface charge at the two pH values. This is not the case for $\text{Al}(\text{OH})_3$, which is positively charged at both pH values and hence shows comparable behaviour in the two sets of tests. This comparison excludes the role of OPA protonation degree and suggests that it is instead the oxide specific electrification that influences adsorption. On the other hand, the interaction of halloysite nanotubes with OPA showed a peculiar character, in terms of adsorption mode and sensitivity to hydrolysis, arising from their complex structure. The present results offer prospects for applications where a selective loading of the inner lumen and a pH-triggered release of active components are required: for example, given that phosphonic acids are retained at pH lower than 4 and released at around pH 7, this system could

prove useful in designing drug delivery devices capable of overcoming the highly acidic gastric environment and release their content upon reaching the intestines, improving the bioavailability of the active ingredient. Moreover, the combined characterization approach proposed here could be applied to other functionalizing agents to clarify their localization and interaction with HNT.

1.4 References

- [1] J. Yang, Y. Wu, Y. Shen, C. Zhou, Y.F. Li, R.R. He, M. Liu, Enhanced Therapeutic Efficacy of Doxorubicin for Breast Cancer Using Chitosan Oligosaccharide-Modified Halloysite Nanotubes, *ACS Appl. Mater. Interfaces*. 8 (2016) 26578–26590. doi:10.1021/acsami.6b09074.
- [2] H. Lun, J. Ouyang, H. Yang, Natural halloysite nanotubes modified as an aspirin carrier, *RSC Adv*. 4 (2014) 44197–44202. doi:10.1039/C4RA09006C.
- [3] Y.M. Lvov, M.M. DeVilliers, R.F. Fakhrullin, The application of halloysite tubule nanoclay in drug delivery, *Expert Opin. Drug Deliv*. 13 (2016) 977–986. doi:10.1517/17425247.2016.1169271.
- [4] P. Yuan, P.D. Southon, Z. Liu, C.J. Kepert, Organosilane functionalization of halloysite nanotubes for enhanced loading and controlled release, *Nanotechnology*. 23 (2012) 375705. doi:10.1088/0957-4484/23/37/375705.
- [5] H. Zhang, T. Ren, Y. Ji, L. Han, Y. Wu, H. Song, L. Bai, X. Ba, Selective Modification of Halloysite Nanotubes with 1-Pyrenylboronic Acid: A Novel Fluorescence Probe with Highly Selective and Sensitive Response to Hyperoxide, *ACS Appl. Mater. Interfaces*. 7 (2015) 23805–23811. doi:10.1021/acsami.5b08600.
- [6] D. Tan, P. Yuan, F. Annabi-Bergaya, D. Liu, L. Wang, H. Liu, H. He, Loading and in vitro release of ibuprofen in tubular halloysite, *Appl. Clay Sci*. 96 (2014) 50–55. doi:10.1016/j.clay.2014.01.018.
- [7] L. Fan, J. Zhang, A. Wang, In situ generation of sodium alginate/hydroxyapatite/halloysite nanotubes nanocomposite hydrogel beads as drug-controlled release matrices, *J. Mater. Chem. B*. 1 (2013) 6261. doi:10.1039/c3tb20971g.
- [8] X. Li, QianYang, J. Ouyang, H. Yang, S. Chang, Chitosan modified halloysite nanotubes as emerging porous microspheres for drug carrier, *Appl. Clay Sci*. 126 (2016) 306–312. doi:10.1016/j.clay.2016.03.035.
- [9] M. Massaro, G. Lazzara, S. Milioto, R. Noto, S. Riela, Covalently modified halloysite clay nanotubes: synthesis, properties, biological and medical applications, *J. Mater. Chem. B*. 5 (2017) 2867–2882. doi:10.1039/c7tb00316a.
- [10] L. Sang, K.M. Knesting, A. Bulusu, A.K. Sigdel, A.J. Giordano, S.R. Marder, J.J. Berry, S. Graham, D.S. Ginger, J.E. Pemberton, Effect of time and deposition method on quality of phosphonic acid modifier self-assembled monolayers on indium zinc oxide, *Appl. Surf. Sci*. 389 (2016) 190–198. doi:10.1016/j.apsusc.2016.06.183.
- [11] D.M. Spori, N. V. Venkataraman, S.G.P. Tosatti, F. Durmaz, N.D. Spencer, S. Zürcher,

Influence of alkyl chain length on phosphate self-assembled monolayers, *Langmuir*. 23 (2007) 8053–8060. doi:10.1021/la700474v.

- [12] S.P. Pujari, L. Scheres, A.T.M. Marcelis, H. Zuilhof, Covalent surface modification of oxide surfaces, *Angew. Chemie - Int. Ed.* 53 (2014) 6322–6356. doi:10.1002/anie.201306709.
- [13] O. Acton, D. Hutchins, L. Árnadóttir, T. Weidner, N. Cernetic, G.G. Ting, T.W. Kim, D.G. Castner, H. Ma, A.K.Y. Jen, Spin-cast and patterned organophosphonate self-assembled monolayer dielectrics on metal-oxide-activated Si, *Adv. Mater.* 23 (2011) 1899–1902. doi:10.1002/adma.201004762.
- [14] S. Lassiaz, D. Labarre, A. Galarneau, D. Brunel, P.H. Mutin, Modification of silica by an organic monolayer in aqueous medium using octylphosphonic acid and aluminium species, *J. Mater. Chem.* 21 (2011) 8199–8205. doi:10.1039/c1jm10128e.
- [15] S.K. Davidowski, G.P. Holland, Solid-State NMR Characterization of Mixed Phosphonic Acid Ligand Binding and Organization on Silica Nanoparticles, *Langmuir*. 32 (2016) 3253–3261. doi:10.1021/acs.langmuir.5b03933.
- [16] P.H. Mutin, V. Lafond, A.F. Popa, M. Granier, L. Markey, A. Dereux, Selective Surface Modification of SiO₂–TiO₂ Supports with Phosphonic Acids, *Chem. Mater.* 16 (2004) 5670–5675. doi:10.1021/cm035367s.
- [17] R.J.P. Corriu, D. Leclercq, P.H. Mutin, L. Sarlin, A. Vioux, Nonhydrolytic sol-gel routes to layered metal(IV) and silicon phosphonates, *J. Mater. Chem.* 8 (1998) 1827–1833. doi:10.1039/a803755h.
- [18] C. Weinberger, T. Heckel, P. Schnippering, M. Schmitz, A. Guo, W. Keil, H.C. Marsmann, C. Schmidt, M. Tiemann, R. Wilhelm, Straightforward Immobilization of Phosphonic Acids and Phosphoric Acid Esters on Mesoporous Silica and Their Application in an Asymmetric Aldol Reaction, *Nanomaterials*. 9 (2019) 249. doi:10.3390/nano9020249.
- [19] F. Hoffmann, M. Cornelius, J. Morell, M. Fröba, Silica-Based Mesoporous Organic–Inorganic Hybrid Materials, *Angew. Chemie Int. Ed.* 45 (2006) 3216–3251. doi:10.1002/anie.200503075.
- [20] A. Vega, P. Thissen, Y.J. Chabal, Environment-controlled tethering by aggregation and growth of phosphonic acid monolayers on silicon oxide, *Langmuir*. 28 (2012) 8046–8051. doi:10.1021/la300709n.
- [21] H. Jing, Y. Higaki, W. Ma, H. Wu, W.O. Yah, H. Otsuka, Y.M. Lvov, A. Takahara, Internally Modified Halloysite Nanotubes as Inorganic Nanocontainers for a Flame Retardant, *Chem. Lett.* 42 (2013) 121–123. doi:10.1246/cl.2013.121.
- [22] D.C.O. Marney, W. Yang, L.J. Russell, S.Z. Shen, T. Nguyen, Q. Yuan, R. Varley, S. Li, Phosphorus intercalation of halloysite nanotubes for enhanced fire properties of polyamide 6, *Polym. Adv. Technol.* 23 (2012) 1564–1571. doi:10.1002/pat.3030.
- [23] C. Breen, N. D’Mello, J. Yarwood, The thermal stability of mixed phenylphosphonic acid/water intercalates of kaolin and halloysite. A TG–EGA and VT–DRIFTS study, *J. Mater. Chem.* 12 (2002) 273–278. doi:10.1039/b104254h.
- [24] W.O. Yah, A. Takahara, Y.M. Lvov, Selective Modification of Halloysite Lumen with Octadecylphosphonic Acid: New Inorganic Tubular Micelle, *J. Am. Chem. Soc.* 134

(2012) 1853–1859. doi:10.1021/ja210258y.

- [25] S.L. Greasley, S.J. Page, S. Sirovica, S. Chen, R.A. Martin, A. Riveiro, J. V. Hanna, A.E. Porter, J.R. Jones, Controlling particle size in the Stöber process and incorporation of calcium, *J. Colloid Interface Sci.* 469 (2016) 213–223. doi:10.1016/j.jcis.2016.01.065.
- [26] B.K. Kang, H.D. Lim, S.R. Mang, K.M. Song, M.K. Jung, S.-W. Kim, D.H. Yoon, Synthesis and Characterization of Monodispersed β -Ga₂O₃ Nanospheres via Morphology Controlled Ga₄(OH)₁₀SO₄ Precursors, *Langmuir*. 31 (2015) 833–838. doi:10.1021/la504209f.
- [27] F. Milanesi, G. Cappelletti, R. Annunziata, C.L. Bianchi, D. Meroni, S. Ardizzone, Siloxane-TiO₂ hybrid nanocomposites. the structure of the hydrophobic layer, *J. Phys. Chem. C*. 114 (2010) 8287–8293. doi:10.1021/jp1014669.
- [28] R. Luschtinetz, G. Seifert, E. Jaehne, H.J.P. Adler, Infrared spectra of alkylphosphonic acid bound to aluminium surfaces, *Macromol. Symp.* 254 (2007) 248–253. doi:10.1002/masy.200750837.
- [29] T. Vallant, J. Kattner, H. Brunner, U. Mayer, H. Hoffmann, Investigation of the formation and structure of self-assembled alkylsiloxane monolayers on silicon using in situ attenuated total reflection infrared spectroscopy, *Langmuir*. 15 (1999) 5339–5346. doi:10.1021/la9900977.
- [30] D. Meroni, S. Ardizzone, U.S. Schubert, S. Hoepfner, Probe-Based Electro-Oxidative Lithography of OTS SAMs Deposited onto Transparent ITO Substrates, *Adv. Funct. Mater.* 22 (2012) 4376–4382. doi:10.1002/adfm.201200673.
- [31] S. Zorn, U. Dettinger, M.W.A. Skoda, R.M.J. Jacobs, H. Peisert, A. Gerlach, T. Chassé, F. Schreiber, Stability of hexa(ethylene glycol) SAMs towards the exposure to natural light and repeated reimmersion, *Appl. Surf. Sci.* 258 (2012) 7882–7888. doi:10.1016/j.apsusc.2012.04.110.
- [32] P.J. Hotchkiss, S.C. Jones, S.A. Paniagua, A. Sharma, B. Kippelen, N.R. Armstrong, S.R. Marder, The modification of indium tin oxide with phosphonic acids: Mechanism of binding, tuning of surface properties, and potential for use in organic electronic applications, *Acc. Chem. Res.* 45 (2012) 337–346. doi:10.1021/ar200119g.
- [33] P. Thissen, A. Vega, T. Peixoto, Y.J. Chabal, Controlled, Low-Coverage Metal Oxide Activation of Silicon for Organic Functionalization: Unraveling the Phosphonate Bond, (2012). doi:10.1021/la3038457.
- [34] P. Kim, S.C. Jones, P.J. Hotchkiss, J.N. Haddock, B. Kippelen, S.R. Marder, J.W. Perry, Phosphonic acid-modified barium titanate polymer nanocomposites with high permittivity and dielectric strength, *Adv. Mater.* 19 (2007) 1001–1005. doi:10.1002/adma.200602422.
- [35] F. Brodard-Severac, G. Guerrero, J. Maquet, P. Florian, C. Gervais, P.H. Mutin, High-field ¹⁷O MAS NMR investigation of phosphonic acid monolayers on titania, *Chem. Mater.* 20 (2008) 5191–5196. doi:10.1021/cm8012683.
- [36] M. Kosmulski, Isoelectric points and points of zero charge of metal (hydr)oxides: 50 years after Parks' review, *Adv. Colloid Interface Sci.* 238 (2016) 1–61. doi:10.1016/j.cis.2016.10.005.

- [37] P.C. Crofts, G.M. Kosolapoff, Preparation and Determination of Apparent Dissociation Constants of Some Alkylphosphonic and Dialkylphosphinic Acids, *J. Am. Chem. Soc.* 75 (1953) 3379–3383. doi:10.1021/ja01110a024.
- [38] E. Joussein, S. Petit, J. Churchman, B. Theng, D. Righi, B. Delvaux, Halloysite clay minerals – a review, *Clay Miner.* 40 (2005) 383–426. doi:10.1180/0009855054040180.
- [39] B.K.G. Theng, Surface Properties of Allophane, Halloysite, and Imogolite, *Clays Clay Miner.* 30 (1982) 143–149. doi:10.1346/CCMN.1982.0300209.
- [40] G. Soliveri, V. Pifferi, R. Annunziata, L. Rimoldi, V. Aina, G. Cerrato, L. Falciola, G. Cappelletti, D. Meroni, Alkylsilane-SiO₂ Hybrids. A Concerted Picture of Temperature Effects in Vapor Phase Functionalization, *J. Phys. Chem. C.* 119 (2015) 15390–15400. doi:10.1021/acs.jpcc.5b04048.
- [41] P. Yuan, D. Tan, F. Annabi-Bergaya, Properties and applications of halloysite nanotubes: recent research advances and future prospects, *Appl. Clay Sci.* 112–113 (2015) 75–93. doi:10.1016/j.clay.2015.05.001.
- [42] G. Soliveri, R. Annunziata, S. Ardizzone, G. Cappelletti, D. Meroni, Multiscale rough titania films with patterned hydrophobic/oleophobic features, *J. Phys. Chem. C.* 116 (2012) 26405–26413. doi:10.1021/jp309397c.
- [43] G. Tari, I. Bobos, C.S.F. Gomes, J.M.F. Ferreira, Modification of surface charge properties during kaolinite to halloysite-7Å transformation, *J. Colloid Interface Sci.* 210 (1999) 360–366. doi:10.1006/jcis.1998.5917.
- [44] G. Cavallaro, G. Lazzara, S. Milioto, Exploiting the Colloidal Stability and Solubilization Ability of Clay Nanotubes/Ionic Surfactant Hybrid Nanomaterials, *J. Phys. Chem. C.* 116 (2012) 21932–21938. doi:10.1021/jp307961q.
- [45] Y. Lvov, W. Wang, L. Zhang, R. Fakhrullin, Halloysite Clay Nanotubes for Loading and Sustained Release of Functional Compounds, *Adv. Mater.* 28 (2016) 1227–1250. doi:10.1002/adma.201502341.
- [46] C. Bretti, S. Cataldo, A. Gianguzza, G. Lando, G. Lazzara, A. Pettignano, S. Sammartano, Thermodynamics of Proton Binding of Halloysite Nanotubes, *J. Phys. Chem. C.* 120 (2016) 7849–7859. doi:10.1021/acs.jpcc.6b01127.

CHAPTER 2

Thiahelicene-grafted halloysite nanotubes: characterization, biological studies and pH triggered release

After the initial study on the selective surface modification of halloysite with phosphonic acids, discussed in the previous chapter, a different strategy was investigated for the pH-driven release of bioactive molecules from covalently modified HNT, based on the use of organosilanes as linkers.

Organosilanes are a family of compounds showing a marked reactivity towards oxides: their silane heads hydrolyse and condensate with the hydroxyl groups on the oxide surface, forming covalent bonds that are stable in a wide range of pH values; their organic tails, which often carry other functional moieties, are exploited to modify surface properties, such as charge and wettability[1], or to provide anchoring sites to link other molecules. In particular, (3-aminopropyl)triethoxysilane (APTES) is frequently employed in the literature to coat oxides with reactive amino groups, which are often reacted with the functional groups of other compounds, including other linkers or bioactive molecules[2,3]. Although halloysite functionalization with APTES has been widely reported in the literature[2], thanks to the oxide-friendly chemistry and ease of use of organosilanes, it is most often used either as a way of inverting the surface charge from negative to positive[4–6] or as a means of grafting macromolecules on the surface of nanotubes to create polymer coatings[3,7–10].

In the present work, a new nanoconstruct based on HNT was prepared in which a bioactive molecule, bearing a formyl group, is linked to the oxide carrier via APTES through the formation of an imine bond. Grafting by an imine bond enabled to trigger the controlled release of the active principle by exposing the nanoconstruct to mild acidic conditions.

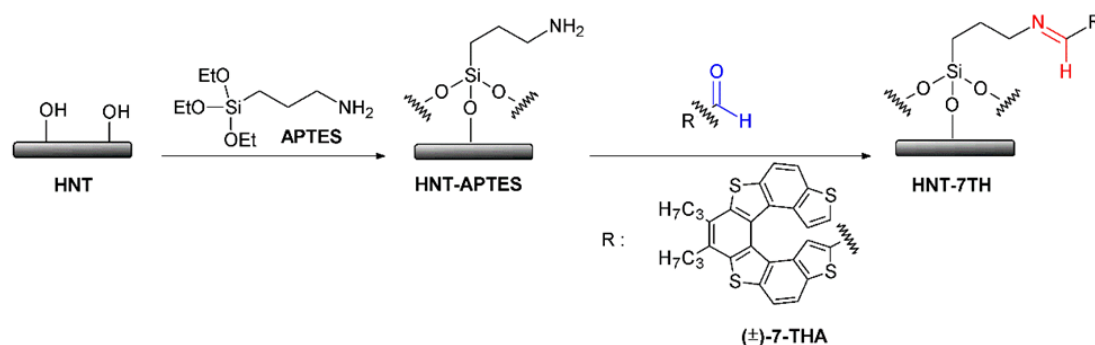
Acid responsiveness is one of the most studied means to elicit the triggered release of bioactive components[11], as it is known that acidic pH is a major feature of extracellular tumour tissue[12] and of lysosomes dedicated to phagocytizing foreign bodies[13].

In the same way, while different pH-sensitive systems based on halloysite nanotubes have been already reported, in most cases the bioactive compound was held onto the nanocarrier solely by electrostatic forces, which grant less control over release kinetics. The release of molecules was then elicited either by the swelling of a polymer matrix[14] or by the surface charge inversion occurring at different pH values[15,16]. A recent paper by Jamshidzadeh and co-authors described the use of pectin and chitosan to prepare pH-sensitive halloysite composites capable of loading and releasing anti-epileptic drugs[17]. They focused on the digestive system as target, hence very

different pH values were investigated (pH 1 and 7.4 to simulate gastric and intestinal pH, respectively), and as their nanosystem relied on electrostatic forces, a massive drug release by diffusion was observed in the first few minutes. On the contrary, the aim of the present investigation was to covalently link the molecule to the support. For this reason, a pH-sensitive drug delivery system based on imine chemistry was designed. Indeed, the imine bond is a suitable tool to achieve the pH-triggered release of biomolecules, which can be integrated in bioorthogonal systems[18].

The active molecule chosen for this preliminary study is ($\pm$)-tetrathia[7]helicene (($\pm$)-7-TH), modified with a formyl group to obtain a tetrathia[7]helicene-based aldehyde (($\pm$)-7-THA)[19], in the form of a racemic mixture. Helicenes are polycyclic compounds in which *ortho*-condensed (hetero)aromatic rings adopt a helical conformation to avoid the overlapping of the terminal rings. Due to their nonplanar π -electron structure, such molecules are inherently chiral and exhibit unique structural and chiroptical properties that have stimulated research in numerous fields[20]. The similarity of helicenes to chiral helical structures found in natural biomolecules, such as DNA and α -helix secondary structures of proteins, has prompted research in biochemistry[21], including studies on potential applications as DNA intercalators[22], as fluorescent dyes for bioimaging[23], and as inhibitors of telomerase[24]. In this respect, the loading of helicene derivatives on nanocarrier systems can enable a more precise localization and targeted delivery of the molecule. However, nanosystems containing helicene derivatives have been scarcely investigated so far, and most of these studies focused on the use of gold[25,26], silica[27] and organic nanoparticles (NPs)[27–29] as carriers. Recently, Cauteruccio et al. have reported the intracellular delivery of a luminescent tetrathia[7]helicene (7-TH) mediated via poly(lactic-co-glycolic acid) (PLGA) NPs[27]. Organic NPs based on fluorescent tetrahydro[5]helicene[28] and squalene-based nanoassemblies containing diaza[4]helicene dyes have also been investigated for cell fluorescence imaging[29]. In these reports, loading is either driven by physical interactions, leading to poor control over the release process, or it is based on stable, non-cleavable covalent bonds such as amide bonds or Au-S interactions with thiol groups.

To covalently graft the helicene derivative to HNT, ($\pm$)-7-THA was reacted with HNT covered with amino groups from APTES (Scheme 2.1); the reversibility of this bond was then tested under slightly different pH conditions, representative of extracellular pH values, to measure the relative release efficiency of 7-TH.



Scheme 2.1 – Reaction pathway for the preparation of HNT-7TH.

2.1 Experimental procedures

2.1.1 Materials

Chemicals were acquired from Sigma-Aldrich (unless differently stated) and were used without further purification; water was doubly distilled and deionised through a Milli-Q apparatus.

2.1.2 Synthesis of tetrathiahelicene and benzodithiophene derivatives

7,8-dipropyl-2-formyltetrathia[7]helicene[19] ($\pm$)-**7-THA** and benzo[1,2-*b*:4,3-*b'*]dithiophen-2-carbaldehyde (**BDTA**)[30] were synthesised by the group of Prof. E. Licandro and Dr. S. Cauteruccio at the Università degli Studi di Milano, according to previous reports. The 7-TH scaffold selected in this study, bearing two *n*-propyl chains on the central benzene ring, is especially convenient from a synthetic point of view, thanks to its efficient, reliable and reproducible synthetic procedure[19]. The purity of ($\pm$)-**7-THA** (up to 97%) and **BDTA** (up to 99%) was evaluated by reverse-phase HPLC analyses that were performed on an Agilent 1100 Series system equipped with DAD analyser, using the analytical column Zorbax EclipseXDB-C18 (150 mm x 4.6 mm x 5 μ m) at a flow rate of 1 mL/min. Acetonitrile and a mixture of acetonitrile/H₂O (9:1) were used as eluent for **7-THA** and **BDTA**, respectively, under isocratic conditions with detection at 254, 300, 350 and 430 nm.

2.1.3 Preparation of HNT-APTES

500 mg of HNT (Hallopure, iMinerals) was suspended in 20 mL of anhydrous toluene and subjected to four vacuum cycles at 200 mbar (3 min each) to remove air trapped in the HNT inner lumen and then left under N₂ atmosphere overnight. After 600 μ L (2.13 mmol) of APTES was added, the suspension was refluxed while stirring for 8 h. Heating was then stopped and the mixture was left to cool overnight. The powder was washed with toluene by centrifugation/resuspension cycles (8 x 35 mL). The solid was then dried in an oven at 80 °C for 24 h.

2.1.4 Preparation of HNT-7TH

300 mg of HNT-APTES was suspended in 20 mL of toluene and subjected to four vacuum cycles at 200 mbar (3 min each), then 150 mg (0.29 mmol) of **7-THA** was dissolved in 10 mL of dichloromethane and added to the suspension. The reaction mixture was stirred for 8 h at 70 °C. The mixture was left to cool overnight. The solid was washed several times with dichloromethane by centrifugation/resuspension cycles (6 x 25 mL) and dried in an oven at 70 °C for 24 h.

2.1.5 Preparation of HNT-BDT

300 mg of HNT-APTES was suspended in 20 mL of toluene; the suspension was sonicated for 5 min and then subjected to four vacuum cycles at 200 mbar (3 min each). 300 mg (1.37 mmol) of benzodithiophene-2-carbaldehyde (**BDTA**) was dissolved in 10 mL of a 9:1 toluene/dichloromethane mixture and added to the suspension. The suspension was stirred for 8 h at 70 °C, then it was left to cool overnight. The solid was washed several times with dichloromethane by centrifugation/resuspension cycles (6 x 25 mL) and dried in oven at 70 °C for 24 h.

2.1.6 Preparation of silica and alumina films

As a model of the outer and inner surface, respectively, silica and aluminium oxide films were prepared for the NEXAFS characterization.

A SiO₂ stable sol was prepared, adding 10 g (48 mmol) of (tetraethyl)orthosilicate (TEOS) to a solution composed of 19.7 mL of ethanol and 4.5 mL of 0.1 M aqueous HCl. After stirring at room temperature for 2 h, the mixture was heated at 60 °C for 1 h. Afterwards, 25 g of a 0.22 M ethanol solution of cetyltrimethylammonium bromide (CTAB) was added.

An aluminium oxide stable sol was prepared by dissolving 2.23 g (11 mmol) of Al(*i*PrO)₃ in 20 mL of water. After stirring for 1 h at 80 °C, 100 mg (1.7 mmol) of glacial CH₃COOH was added and the solution was stirred at 80 °C for 8 h.

Silica and aluminium oxide films were then prepared by dip coating a 1x1 cm² FTO covered glass (resistivity ~7 Ω/sq), which was then calcined at 300 °C (4 h, O₂ flux at 9 L/h).

2.1.7 Functionalization of films

Previously prepared silica and aluminium oxide films were immersed in 10 mL of anhydrous toluene under N₂ atmosphere. 330 μL (1.41 mmol) of APTES was added to the suspension, which was refluxed for 8 h. The reaction mixture was allowed to cool overnight. Films were then washed several times with toluene (8x35 mL) using an ultrasonic bath. They were then dried in oven at 80 °C for 24 h.

APTES-functionalized films were immersed in 10 mL of a toluene/ dichloromethane 2:1 mixture. 15 mg (0.029 mmol) of 7-THA was then added and the solution was kept at 70 °C for a total of 16 h, to compensate for the lack of stirring. At the end of the reaction, films were washed with dichloromethane (6x35 mL) in an ultrasonic bath and dried in oven at 70 °C for 24 h.

2.1.8 Sample characterization

Thermogravimetric Analysis (TGA) curves were acquired using a Mettler-Toledo TGA/DSC 3+ STAR System, between 30 and 900 °C with a rate of 3 °C min⁻¹. TGA analyses were conducted under air flux.

Powder X-ray Diffraction (PXRD) patterns of HNT samples were acquired using a Siemens D5000 diffractometer equipped with a Cu K α source (λ = 0.15406 nm), working at room temperature and 40 kV x 40 mA nominal X-rays power. θ : 2θ scans were registered between 10° and 80°, with a step of 0.02°.

The specific surface areas of the samples were determined by N₂ adsorption-desorption isotherms in subcritical conditions recorded on a Coulter SA 3100 apparatus and elaborated by the Brunauer–Emmett-Teller (BET) method.

The HNT morphology was evaluated via transmission electron microscopy (TEM) using a Zeiss LEO 912ab Energy Filtering microscope operating at 120 kV and equipped with a CCD-BM/1 K system.

Fourier Transform Infrared (FTIR) spectra were registered using a Perkin Elmer Spectrum 100 spectrophotometer in Attenuated Total Reflectance (ATR) mode, acquiring 12 scans between 4000 and 400 cm⁻¹ with a resolution of 4.0 cm⁻¹.

Near Edge X-ray Absorption Fine structure (NEXAFS) experiments were performed at the Materials Science Beamline at the Elettra Synchrotron (Basovizza, IT). The light source of the beamline is a bending magnet and the beamline is equipped with a plane grating monochromator able to tune photon energy from 22 to 1000 eV, and a hemispherical analyser for electron energy (Specs Phoibos 150). The main chamber had a pressure of 10⁻⁹ mbar.

Nitrogen K-edge and carbon K-edge NEXAFS spectra were acquired both at normal (NI, 90°) and grazing (GI, 10°) incidence of the photon beam relative to the surface. Beam polarization was assumed to be 80-90% linear. Signal normalization was carried out, so that the pre-edge baseline is zero and the post edge intensity is 1.

X-ray Photoelectron Spectroscopy (XPS) was performed with an M-probe apparatus (Surface Science Instruments). The source was monochromatic Al K α radiation (1486.6 eV). The C 1s peak was referenced to 284.8 eV to correct the binding energies (BE) for sample charging, and the background was subtracted using Shirley's method. The fittings were performed using a combination of Lorentzian and Gaussian line shapes. The accuracy of the reported BE can be estimated to be ± 0.3 eV.

2.1.9 Release tests

Buffer solutions at pH 5, 6.8 and 7.4 were used. The first was a commercial citrate buffer (Sigma-Aldrich), while the other two were phosphate-based and prepared in-house: to obtain a pH 6.8 buffer, 51 mL of a 0.2 M KH₂PO₄ aqueous solution was mixed with 49 mL of a 0.2 M Na₂HPO₄ aqueous solution, then diluted to 200 mL. The pH 7.4 buffer was prepared analogously, by mixing 19 mL of a 0.2 M KH₂PO₄ aqueous

solution with 81 mL of a 0.2 M Na₂HPO₄ aqueous solution, then diluted to 200 mL. the concentration of all three buffers was 0.1 M.

2.1.9.1 Functionalized powders

50 mg of HNT-BDT was suspended in 10 mL of a 1% THF mixture of each buffer solution using an ultrasonic probe. Once suspended, the powder was stirred continuously for 48 h, sampling 2 mL after 1, 4, 7, 24 and 48 h. Each sample was centrifuged to remove the solid phase. The supernatant was analysed via UV-vis spectroscopy, measuring the absorbance at 340 nm with a Shimadzu UV2600 spectrophotometer.

2.1.9.2 Functionalized films

Silica and alumina films functionalized with **7-THA** were immersed in 10 mL of a citrate buffer solution (pH 5). After 48 hours, the supernatant was removed, films were washed with water and dried in air.

2.1.10 Cell culture and cell viability assay

Cell viability assays were carried out by Dr. R. Vago at the IRCSS San Raffaele Scientific Institute (Milan, Italy).

5637 and HT-1376 human bladder cancer cells were maintained in RPMI 1640 medium supplemented with 10% (v/v) foetal bovine serum, 50 U/ml penicillin and 50 µg/ml streptomycin at 37 °C in a 5% CO₂ humidified atmosphere.

To test the effect of helicene containing HNT on cell viability, cells were plated in 96-well plates at a cell density of 5×10^3 cells/well and treated with serial logarithmic dilutions of HNT-APTES or HNT-APTES-7TH ranking from 0.5 µM to 0.05 nM for 72 h. Control cultures received the respective volume of DMSO. At the end of the exposure period, MTT assay was performed according to the manufacturer's recommendations (Sigma-Aldrich). Formazan product was resuspended in DMSO and measured in a spectrophotometric microplate reader at 570 nm wavelength. Each experiment was performed in quadruplicate and data were reported as mean ± standard deviation. Cell viability was evaluated as the concentration inhibiting the 50% of growth with respect to untreated control cells and is expressed as IC₅₀.

2.2 Results and discussion

2.2.1 Structure and morphology of halloysite nanotubes

In this first study, commercial HNT from iMinerals Inc. was used, as received. Diffractograms show halloysite as the main phase, with kaolinite and minor quartz impurities (Figure 2.1). The pristine HNT has a specific surface area of $33.7 \text{ m}^2 \text{ g}^{-1}$, with a total pore volume of 0.204 mL g^{-1} . This is mostly represented by the inner lumen of the nanotubes, as the pores with a diameter of 20-80 nm account for around 60% of the total pore volume, in good agreement with TEM images (Figure 2.2).

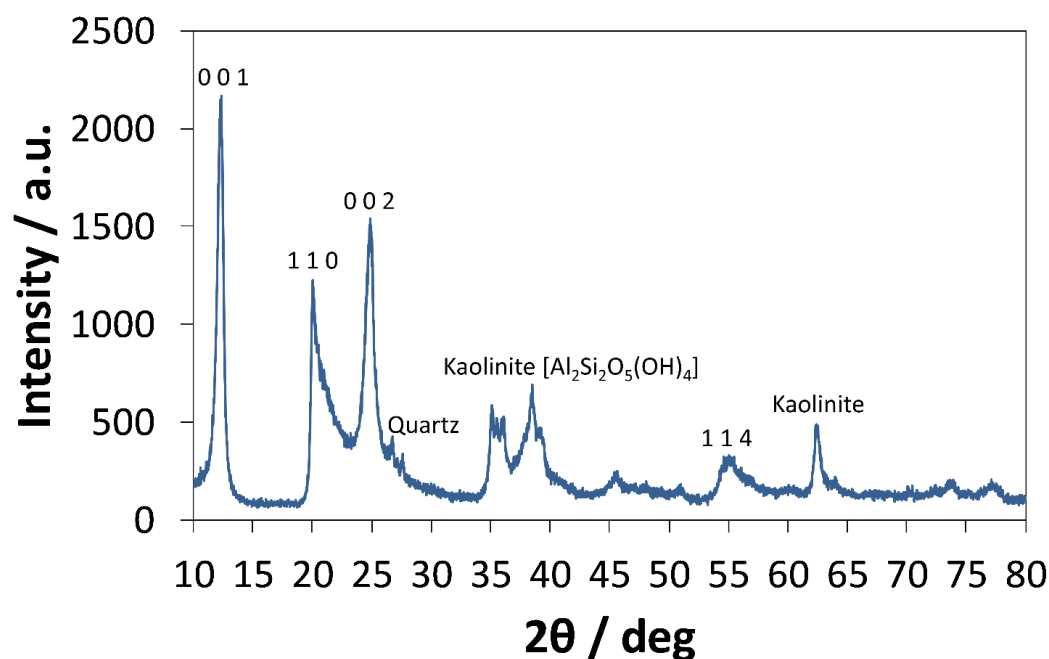


Figure 2.1 – PXRD pattern of pristine HNT. The main reflection of halloysite phase are indexed[30] and the attribution is reported for the peaks of main crystalline impurities.

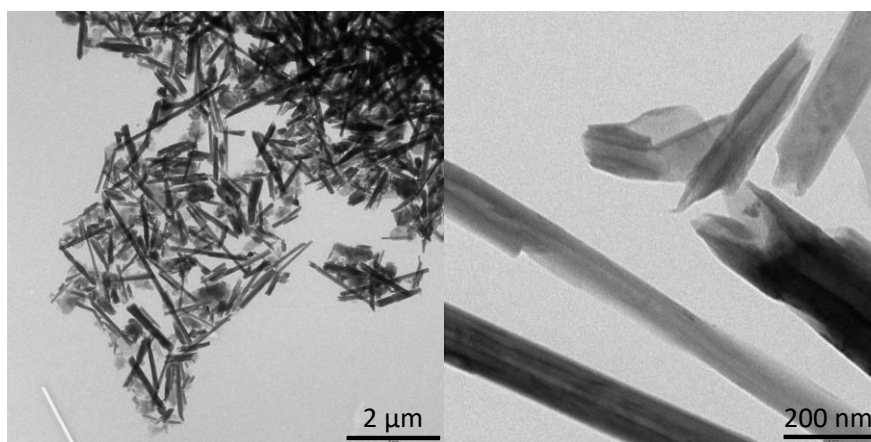


Figure 2.2 – TEM image of the as received HNT sample.

2.2.2 TGA results

TGA analysis was used to calculate the surface grafting upon functionalization with APTES and loading of 7-TH. Figure 2.3 reports the TGA curves of both the pristine, APTES functionalized and 7-TH grafted HNT. The bare HNT sample shows three characteristic weight losses below 100 °C, around 250 °C and 450 °C, which can be attributed to the loss of physisorbed water, interlayer water and dehydroxylation of the inner surface, respectively[31]. Upon modification with APTES, a weight loss at around 300 °C becomes appreciable, which can be attributed to the degradation of the alkyl chain of APTES. After functionalization with **7-THA**, another component appears above 500 °C, which can be related to the degradation of the aromatic substituent.

The loading percentage of 7-TH was calculated as the ratio between the loaded molecule mass over the mass of the 7-TH-loaded HNT powder. The 7-TH loaded amount was estimated from the mass loss centred at *ca.* 600 °C, which is present only in the HNT-7TH sample and can thus be related to the degradation of the aromatic substituent. A 7-TH loading of 3.5% was determined, in good agreement with previous study about covalently linked molecules on nanotube systems[18], while much higher loadings have instead been reported in the case of electrostatic encapsulation of drugs in HNT[6,17].

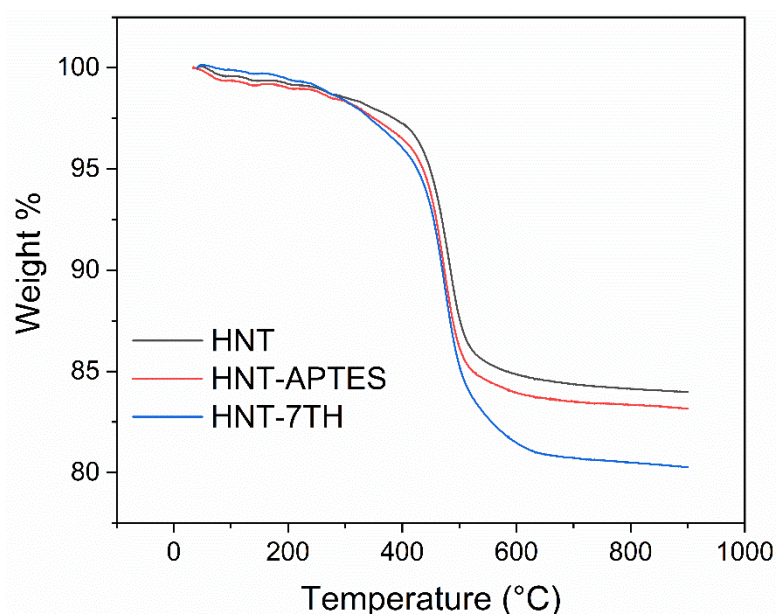


Figure 2.3 – TGA curves of bare HNT, HNT-APTES and HNT-7TH

By correlating these results with the specific surface area of pristine HNT, a rough estimate of the molecule surface coverage δ can be obtained. As APTES possesses three ethoxy moieties, during condensation it can form a variable number of bonds (from 1 to 3) with the oxide surface or neighbouring APTES molecules[32]; hence, knowing exactly what is the molecular weight to consider during thermal degradation is rather difficult[33]. In the present case, the δ value of HNT-APTES can thus range from 1.4 to 2.8 molecules/nm² depending on whether a single bond formation or a full condensation

are assumed, averaging at 2 molecules/nm²: these values compare well with literature data concerning APTES monolayers on glass and silicon substrates (2.1-4.2 molecules/nm²)[34,35]. To calculate these values, the following procedure was adopted.

In the HNT-APTES sample, the weight loss corresponding to the degradation of the APTES alkyl chains accounts for 0.9%w. If we consider (for the sake of clarity) a full condensation of the three ethoxy groups upon APTES grafting, the weight loss would be related merely to the amino-substituted propyl chain (MW 58.08 g mol⁻¹). Hence, the moles of grafted APTES in 100 g of sample can be calculated by the following equation:

$$n_{\text{APTES}} = 0.9 \text{ g} / 58.08 \text{ g mol}^{-1} = 15.5 \text{ mmol}$$

As the grafted mass of APTES includes also the silanol headgroup, the MW of the fully grafted APTES moiety is 134 g mol⁻¹. As a result, the actual mass of HNT in 100 g of sample is:

$$m_{\text{HNT}} = 100 \text{ g} - (15.5 \text{ mmol} \cdot 134.08 \text{ g mol}^{-1}) = 97.9 \text{ g}$$

According to BET analysis, HNT shows a specific surface area (SSA) of 33.7 m² g⁻¹. Surface densities δ , expressed in molecules nm⁻², can then be obtained according to the following equation:

$$\delta_{\text{APTES}} = \frac{n_{\text{APTES}} \cdot N_A}{\text{SSA}_{\text{HNT}} \cdot m_{\text{HNT}} \cdot 10^{18}} = 2.8 \text{ molecules nm}^{-2}$$

where N_A is the Avogadro's number.

If a lower condensation state of APTES is considered, a higher MW of the degraded alkyl chain has to be adopted, leading to lower surface density values (in the range 1.4-2.8 molecules nm⁻²).

In the case of HNT-7TH sample, the weight loss corresponding to the degradation of the 7-TH moiety (MW 514.07 g mol⁻¹) accounts for 3.5%w. The moles of grafted 7-TH can thus be estimated by the following equation:

$$n_{\text{7TH}} = 3.5 \text{ g} / 514.07 \text{ g mol}^{-1} = 6.8 \text{ mmol}$$

Besides the 7-TH moieties, we should also consider the grafted APTES, which we can consider fully grafted for the sake of simplicity. As a result, the actual mass of HNT in 100 g of sample is:

$$m_{\text{HNT}} = 100 \text{ g} - 3.5 \text{ g} - 2.1 \text{ g} = 94.4 \text{ g}$$

The surface density of 7TH molecules can thus be estimated by the following equation:

$$\delta_{\text{7TH}} = \frac{n_{\text{7TH}} \cdot N_A}{\text{SSA}_{\text{HNT}} \cdot m_{\text{HNT}} \cdot 10^{18}} = 1.3 \text{ molecules nm}^{-2}$$

The δ value for 7-TH is thus estimated to be slightly lower than the one for APTES molecules. Since the calculated co-area for standing and lying 7-TH molecules is about 0.6 nm² and 1.25 nm² respectively[36], the δ value supports an average vertical orientation of the helicene moieties at the surface, as further confirmed by the NEXAFS spectra (see Section 2.2.4).

Due to their natural origin, the HNT physicochemical properties can vary even within the same batch. Moreover, their structural and morphological properties exhibit substantial differences across different geological deposits: phase impurity, specific surface area, size polydispersity, aspect ratio, and surface charge are all affected by the specific extraction site of the HNT sample[31], as it will be discussed in detail in the next chapter. Even though HNT from homogeneous source were here employed, the HNT morphology within the adopted batch displays polydispersity in the aspect ratio and surface defects (see Figure 2.2). The surface coverage data here estimated are thus to be considered as average values over the investigated HNT population.

2.2.3 XPS results

After functionalization, HNT-7TH was treated in mild acidic conditions in order to elicit the release of the 7TH moiety: XPS analyses were carried out at each stage of the whole preparation process of HNT-7TH and after the release test at pH 5, to monitor the presence and release of adsorbates on the surface. Results are summarized in Table 2.1. As expected, the pristine HNT surface is characterized by the presence of Al, Si and O, together with some adventitious C (Figure 2.4A). Binding energies of Al (74.9 and 76.2 eV) and Si (103.1 and 104.4 eV) peaks are in line with the literature[37], and could be attributed to M-O and M-OH groups, respectively. Although the stoichiometric ratio between Al and Si should be 1:1, the latter appears to be more abundant, due to attenuation of the inner layer signal[38]. After APTES functionalization a few changes can be noticed: the Si signal presents a lower energy peak at 102.0 eV (Figure 2.4B), which can be attributed to the siloxanes from APTES[32], while the bulk SiO₂ component at 103.1 eV becomes attenuated[39]. The carbon content increases due to the carbon chains of the organic molecule.

Element	HNT	HNT-APTES	HNT-7TH	HNT-7TH pH 5
Al	11.9	7.9	5.7	2.7
Si	14.8	13.3	9.7	5.9
S	-	-	3.3	0.6
C	16.6	36.9	50.7	59.1
N	-	3.5	3.2	1.9
O	56.7	38.4	27.4	29.8

Table 2.1 – Surface atomic percentage amounts of the various samples, obtained by XPS.

Moreover, a broad N signal becomes appreciable (Figure 2.5) in agreement with the literature, showing for APTES-modified oxides the occurrence of two peak components relative to free and hydrogen-bonded NH₂[32]. The N/Si atomic ratio is 0.26, so there is approximately one N atom every four Si atoms at the sample surface. Assuming that the N atom and one of the Si atoms belong to an APTES molecule, this means that there is one APTES molecule every three surface Si atoms. Based on halloysite structural data[40], there are 9 Si atoms/nm² on the external surface. It can thus be concluded that, according to XPS analyses, the APTES molecular density δ is around 3 molecules/nm²,

which is actually within the range determined by TGA analyses, indicating a fully condensed APTES layer.

Compared to HNT-APTES, HNT-7TH shows a further increase in the C content. Additionally, the two peaks of S 2p are appreciable (Figure 2.4C): the binding energy of the 2p_{3/2} component (163.8 eV) is attributable to the thiophene in the tetrathiahelicene scaffold[41], indicating that 7-TH was successfully bound to the surface. Slight changes can also be noticed in the shape of the N signal: after functionalization with **7-THA**, the shape of the peak changes, highlighting an increase in the component at lower binding energy. The latter could be related to the formation of an imine C=N bond, since its signal coincides with that of free NH₂[42,43].

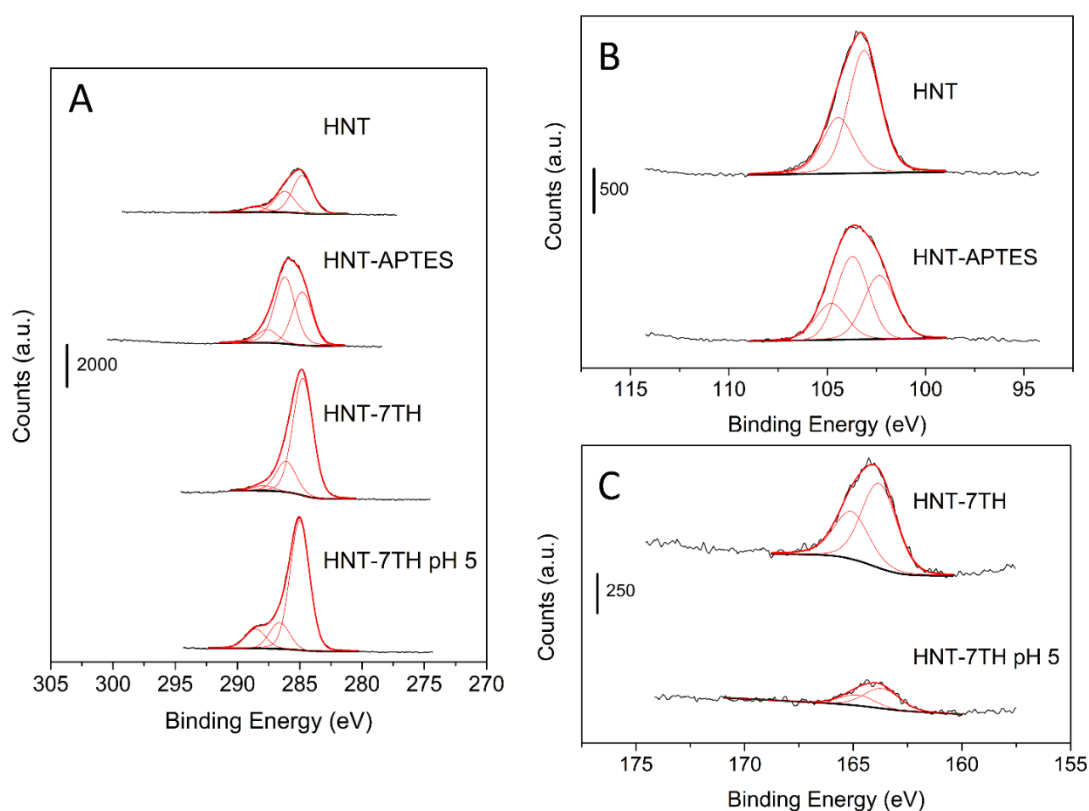


Figure 2.4 – High resolution XPS spectra, with related peak fitting, of HNT, HNT-APTES, HNT-7TH and HNT-7TH after release at pH 5: C 1s (A), Si 2p (B) and S 2p (C) regions.

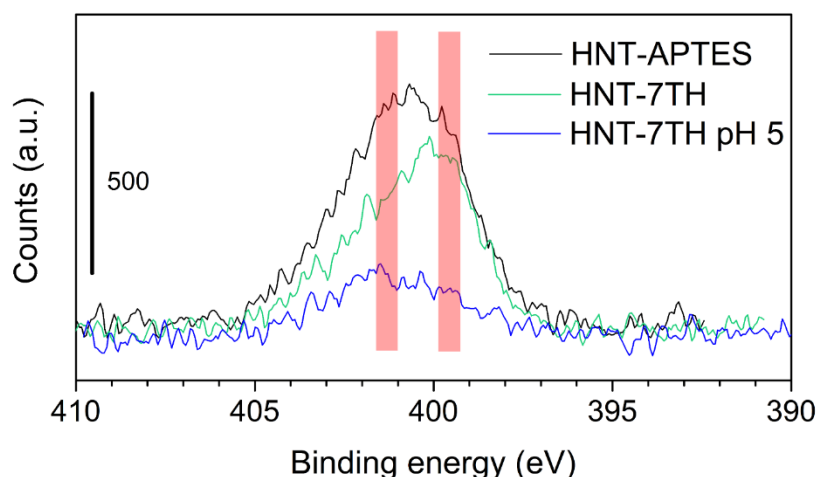


Figure 2.5 – High resolution XPS spectra of the N 1s region. Red bars highlight literature B.E. of free NH_2 (399.6 ± 0.3 eV) and H bonded NH_2 or NH_3^+ (401.3 ± 0.3 eV)[32].

After 48 h of treatment at pH 5, the amount of S appears greatly reduced: the S : N atomic ratio for HNT-7TH goes from 1.03 to 0.33 upon treatment, indicating a loss of almost 70% of the loaded molecule. The carbon and oxygen amounts increase, due to the adsorption of citrate species present in the buffer solution used for the release test, attenuating the N 1s signal. The latter becomes more similar to the HNT-APTES sample (Figure 2.5), indicating the breaking of the imine bond. Furthermore, the presence of a charged species such as citrate ions might favour the NH_3^+ form of APTES, possibly explaining the slight increase in its component in the N 1s signal, as shown in Figure 2.5.

2.2.4 NEXAFS results

To further investigate the surface reactivity of bonded 7-TH, the molecular orientation of the functionalizing moieties at the oxide surface was also investigated via angle-resolved NEXAFS spectroscopy. For this purpose, as models of the HNT surface, silica and aluminium oxide films were prepared, functionalized first with APTES, then with 7-TH and subjected to the same treatment at pH 5 for 48 h. As APTES functionalization is able to bind to both halloysite surfaces, this chapter will refer to the inner alumina layer, as the binding in the inner lumen leads to a more controlled release. Fully comparable results were obtained for the SiO_2 film.

C K-edge and N K-edge NEXAFS spectra of the bare and functionalized films were collected at grazing and normal incidence (Figure 2.6). With respect to a previous study on APTES-functionalized TiO_2 [32], no preferential orientation of the alkyl chains was found in the presently investigated APTES-functionalized oxide films, supporting the formation of a disorganized organic layer. On the contrary, upon reaction with the helicene derivative, preferential orientation of the organic molecules was observed for both sets of substrates: in particular, the $\text{C } 1s \rightarrow \pi^*(\text{C}=\text{C}, \text{C}=\text{N})$ component at 286.0 eV, related to the conjugated ring structure[44], and the $\text{N } 1s \rightarrow \pi^*(\text{N}=\text{C})$ and $\text{N } 1s \rightarrow$

$\sigma^*(\text{N—C})$ components at 399.6 eV and 407.2 eV, respectively[43], suggested a preferential orientation of the aromatic rings of helicene normal to the oxide surface, possibly as a result of π - π stacking interactions. This occurrence may prove useful in promoting drug loading.

Interestingly, NEXAFS spectra show no preferential orientation of the organic chains after the treatment in mild acidic conditions. Furthermore, the $\text{C } 1s \rightarrow \pi^*(\text{C}=\text{C}, \text{C}=\text{N})$ transitions become negligible, while a component appears at 289.2 eV in C K-edge spectra, which can be attributed to the $1s \rightarrow \pi^*(\text{C}=\text{O})$ transition[45] of the citrate anions from the buffer solution, possibly interacting with the protonated amine group formed upon the breaking of the imine bond.

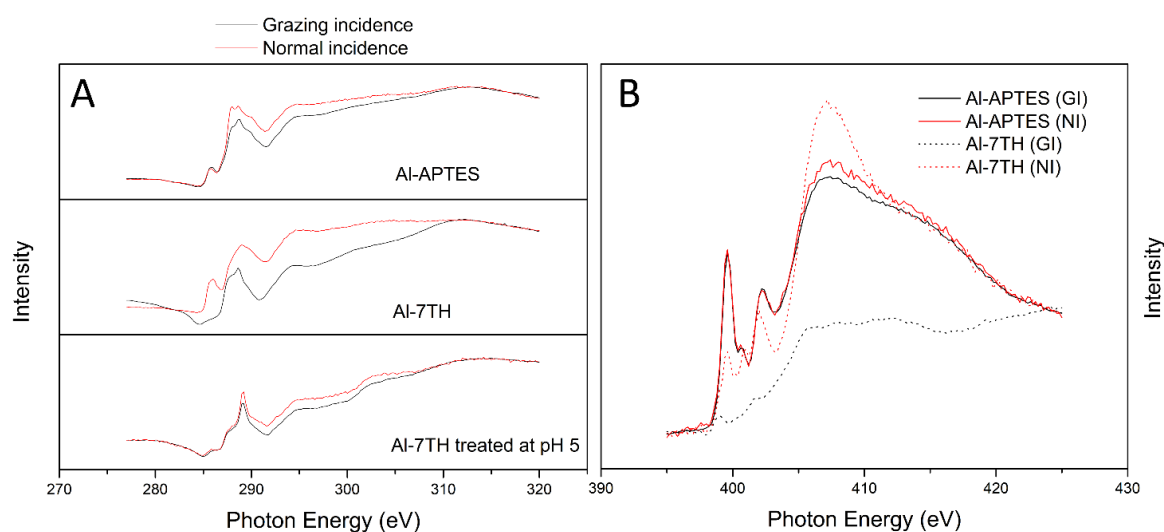


Figure 2.6 – C K-edge (A) and N K-edge (B) angle-resolved NEXAFS spectra of the functionalized aluminium oxide films.

2.2.5 Cell viability tests

The effect of HNT-APTES or HNT-7TH on the viability of bladder cancer cells was tested. To address tumour heterogeneity, tests were carried out on two different cell lines, named 5637 and HT-1376, which differ in grading, proliferation rate and metabolism, with the latter ones being more prone to acidifying the culture medium. Cell viability was assessed by incubating cells with serial logarithmic dilutions of HNT-APTES or HNT-7TH ranking from 0.5 μM to 0.05 nM for 72 h and then a MTT viability assay was performed (Figure 2.7A).

HNT-APTES sample was slightly toxic for cells at the highest tested concentrations in both the cell models. HNT-7TH showed overlapping activity with HNT-APTES on 5637 cells, while it was found to be significantly more active on HT-1376. This result is in line with the acidification propensity of the two cell lines: at the end of the experiment, 5637 culture medium had pH 7.2, while HT-1376 medium was more acidic with pH 6.8. In fact, 7-TH is expected to be released more rapidly from the HNT at slightly acidic pH, as demonstrated by *in vitro* assays, by the breakdown of the imine

bond. The free 7-TH was then able to exert its cytotoxic activity onto HT-1376, causing a reduction of cell viability. This was not the case for 5637 cells, due to the pH of the culture medium, not compatible with 7-TH release. This approach can be valuable for the treatment of tumours that show a strong metabolic rewiring, stimulating aerobic glycolysis instead of respiration, resulting in the concomitant secretion of lactate.

2.2.6 Release kinetics

Since it was confirmed that 7-TH was successfully loaded and released from HNT, further studies on the release kinetics and its dependence on environmental pH were performed. Tests were carried out on a more water-soluble mimic, benzo[1,2-*b*:4,3-*b'*]dithiophene (BDT), which represents a suitable portion of 7-TH. Its formyl derivative is expected to behave very similarly to 7-TH from a reactivity point of view[30]. Release tests were performed by suspending HNT-BDT in three different buffer solutions and monitoring the release for 48 h. Figure 2.7B reports the results of these tests as cumulative release against time. Release efficiency was highest for the treatment at pH 5, reaching maximum BDT solubility after only 7 h. On the contrary, release is much slower in the case of higher pH: at pH 7.4 the amount of released BDT is negligible even after 48 h, while decreasing the pH value by just 0.6 units to 6.8 visibly increases the release rates.

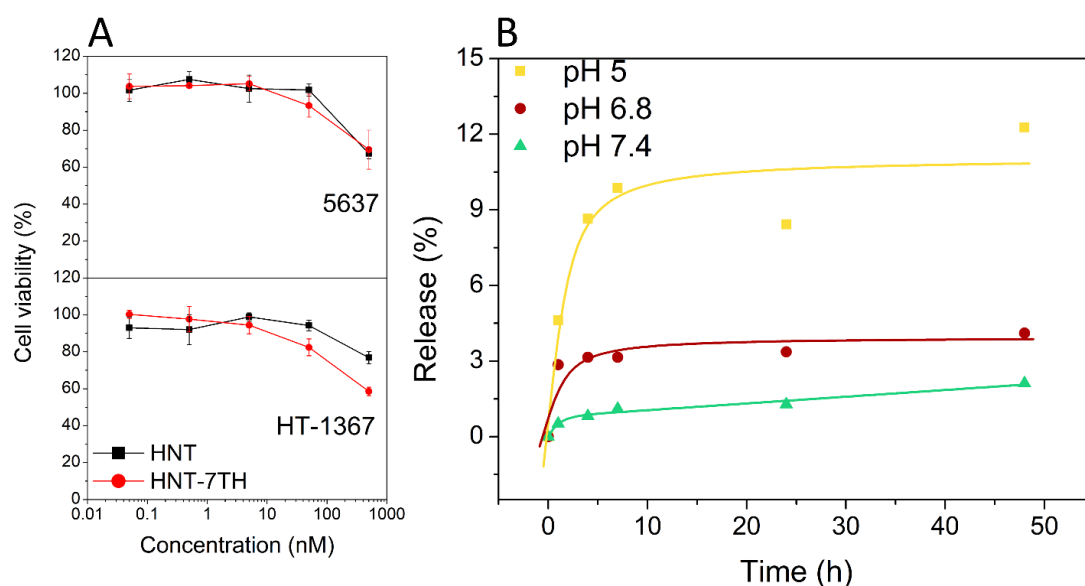


Figure 2.7 – Cell viability assays on 5637 and HT-1367 cell lines (A) and BDT release kinetics under different pH conditions (B).

Drawing a comparison with literature data is complicated since numerous literature reports compare extreme pH conditions, like those of gastric and intestinal fluids[17,18,46], whereas in the present case a narrower pH range was investigated as this work focused on the response of our system to extracellular environments: from pH

7.4 of physiological fluids, to pH 6.8 characteristic of extracellular environment of acidifying cell lines, and pH 5 of lysosomes.

Notwithstanding the differences both in the pH range investigated and in the nature of the nanoconstructs, the present results are in good agreement with the work by Massaro and coauthors[18], who investigated the release of curcumin linked by imine bond in conditions mimicking gastric and intestinal fluids. They reported only about 5% of curcumin release at pH 7.4, showing a relative stability of imine linkages in neutral pH environment, whereas a complete release was observed at pH 1.0, which was attributed to both the pH-sensitivity of the imine bond and to the higher solubility of curcumin in acidic conditions. In the present case, the higher cumulative release observed at pH 6.8 and 5.0 cannot be explained in terms of a promoted solubility in acidic conditions, due to the structure of the 7-TH derivative, and should be solely related to the imine bond reversibility in mildly acidic conditions. The observed lower cumulative release in acidic environments of the helicene derivative, compared to curcumin, could instead be related to the poorer water solubility of the former.

In order to better understand the release kinetics and compare our data with literature reports, release curves were fitted according to the Korsmeyer-Peppas model, which is commonly employed to rationalize the release mechanism in halloysite-based drug delivery systems[2,18]. The following equation was adopted:

$$Q = \frac{M_t}{M_\infty} = kt^n$$

where Q is the drug fraction released at time t , k is the rate constant and n is the diffusion exponent, related to the release mechanism and to the geometry of the carrier. The k and n values were calculated by linearizing the equation and fitting via linear regression.

A clear pH effect is noticeable when comparing k and n values across the three experiments: at normal physiological pH, that is 7.4, release is very slow and diffusion driven ($k = (1.5 \pm 0.4) \times 10^{-3} \text{ min}^{-1}$, $n = 0.3$), while a slight pH decrease to 6.8 (*i.e.*, that of HT-1376 cells), is enough to considerably improve the release kinetics ($k = (24 \pm 3) \times 10^{-3} \text{ min}^{-1}$, $n = 0.08$). A further pH decrease, down to 5, speeds up the release even more ($k = (30 \pm 10) \times 10^{-3} \text{ min}^{-1}$, $n = 0.2$). The observed k values are compatible with a sustained release by imine hydrolysis[18]. Even though n values are lower than 0.45, which is the lower threshold for Fickian diffusion release according to the original model, similar values are generally reported in the case of non-spherical devices[32,33,51,52].

2.2.7 FTIR results

Furthermore, FTIR spectroscopy was performed on HNT-BDT before and after release at pH 5, 6.8 and 7.4. Figure 2.8 reports the differential spectra, for a wavenumber range between 1800 and 1400 cm^{-1} , with respect to HNT-APTES. The main feature of the HNT-BDT spectrum is the peak at 1632 cm^{-1} , which can be attributed to the imine bond, formed upon the reaction between the NH_2 group of APTES and the formyl group of BDT. Upon release tests, other features become appreciable: the sample treated at pH 5

shows strong and broad signals due to the presence of citrate (from the buffer), obscuring other signals. For samples treated at pH 7.4 and 6.8 both the imine signal and the water bending mode at 1650 cm^{-1} are appreciable, with different relative intensities. As can be seen, the relative intensity of the two peaks gets closer when decreasing the release pH, suggesting that there is less imine on the surface, thus supporting the breaking of the imine bond: at pH 7.4 the peaks are very similar to the untreated sample, whereas at 6.8 their intensities are fully comparable. These results support those obtained through *in vitro* studies, which suggested that a small difference in cellular environment pH could be responsible for the higher cytotoxicity observed.

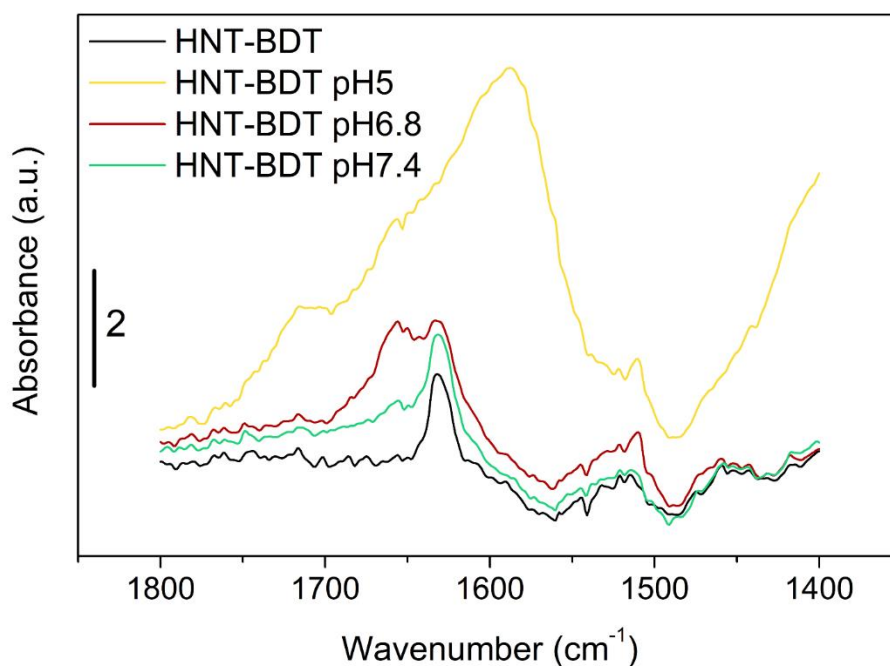


Figure 2.8 – FTIR differential spectra of HNT-BDT after release experiments at various pH values; the spectrum of HNT-APTES was adopted as reference.

2.3 Conclusions

In conclusion, a new nanosystem where tetrathia[7]helicene is grafted via an imine bond to APTES-modified halloysite was developed. The success of the adopted functionalization approach was demonstrated via a combination of spectroscopic techniques, which allowed to gain a better understanding of the packing of the helicene moieties grafted at the surface: the surface density of 7-THA calculated from TGA data suggested that the molecules were vertically oriented, which was confirmed by angle-resolved NEXAFS spectroscopy. The triggered release of thiahelicene was demonstrated: collected spectroscopic evidence supported the helicene release under mildly acidic conditions. Release kinetics tests showed a clear pH dependence, also confirmed by preliminary *in vitro* cell viability tests on two tumour cell lines with different extracellular pH values. Therefore, these systems proved promising candidates

for the triggered release of this cytotoxic compound, with potential therapeutic applications.

2.4 References

- [1] L. Rimoldi, T. Taroni, D. Meroni, Wetting Modification by Photocatalysis: A Hands-on Activity To Demonstrate Photoactivated Reactions at Semiconductor Surfaces, *J. Chem. Educ.* (2018) acs.jchemed.8b00362. doi:10.1021/acs.jchemed.8b00362.
- [2] M. Massaro, G. Lazzara, S. Milioto, R. Noto, S. Riela, Covalently modified halloysite clay nanotubes: synthesis, properties, biological and medical applications, *J. Mater. Chem. B.* 5 (2017) 2867–2882. doi:10.1039/c7tb00316a.
- [3] M. Liu, Y. Chang, J. Yang, Y. You, R. He, T. Chen, C. Zhou, Functionalized halloysite nanotube by chitosan grafting for drug delivery of curcumin to achieve enhanced anticancer efficacy, *J. Mater. Chem. B.* 4 (2016) 2253–2263. doi:10.1039/C5TB02725J.
- [4] P. Yuan, P.D. Southon, Z. Liu, M.E.R. Green, J.M. Hook, S.J. Antill, C.J. Kepert, Functionalization of halloysite clay nanotubes by grafting with γ -aminopropyltriethoxysilane, *J. Phys. Chem. C.* 112 (2008) 15742–15751. doi:10.1021/jp805657t.
- [5] D. Tan, P. Yuan, F. Annabi-Bergaya, H. Yu, D. Liu, H. Liu, H. He, Natural halloysite nanotubes as mesoporous carriers for the loading of ibuprofen, *Microporous Mesoporous Mater.* 179 (2013) 89–98. doi:10.1016/j.micromeso.2013.05.007.
- [6] H. Lun, J. Ouyang, H. Yang, Natural halloysite nanotubes modified as an aspirin carrier, *RSC Adv.* 4 (2014) 44197–44202. doi:10.1039/C4RA09006C.
- [7] C. Li, J. Liu, X. Qu, B. Guo, Z. Yang, Polymer-modified halloysite composite nanotubes, *J. Appl. Polym. Sci.* 110 (2008) 3638–3646. doi:10.1002/app.28879.
- [8] Y. Joo, Y. Jeon, S.U. Lee, J.H. Sim, J. Ryu, S. Lee, H. Lee, D. Sohn, Aggregation and stabilization of carboxylic acid functionalized halloysite nanotubes (HNT-COOH), *J. Phys. Chem. C.* 116 (2012) 18230–18235. doi:10.1021/jp3038945.
- [9] S. Kumar-Krishnan, A. Hernandez-Rangel, U. Pal, O. Ceballos-Sanchez, F.J. Flores-Ruiz, E. Prokhorov, O. Arias de Fuentes, R. Esparza, M. Meyyappan, Surface functionalized halloysite nanotubes decorated with silver nanoparticles for enzyme immobilization and biosensing, *J. Mater. Chem. B.* 4 (2016) 2553–2560. doi:10.1039/C6TB00051G.
- [10] S.S. Zargarian, V. Haddadi-Asl, H. Hematpour, Carboxylic acid functionalization of halloysite nanotubes for sustained release of diphenhydramine hydrochloride, *J. Nanoparticle Res.* 17 (2015) 218. doi:10.1007/s11051-015-3032-3.
- [11] S.R. MacEwan, D.J. Callahan, A. Chilkoti, Stimulus-responsive macromolecules and nanoparticles for cancer drug delivery, *Nanomedicine.* 5 (2010) 793–806. doi:10.2217/nnm.10.50.
- [12] Y. Kato, S. Ozawa, C. Miyamoto, Y. Maehata, A. Suzuki, T. Maeda, Y. Baba, Acidic

extracellular microenvironment and cancer, *Cancer Cell Int.* 13 (2013) 89. doi:10.1186/1475-2867-13-89.

- [13] J.E. DiCiccio, B.E. Steinberg, Lysosomal pH and analysis of the counter ion pathways that support acidification, *J. Gen. Physiol.* 137 (2011) 385–390. doi:10.1085/jgp.201110596.
- [14] K.M. Rao, S. Nagappan, D.J. Seo, C.S. Ha, PH sensitive halloysite-sodium hyaluronate/poly(hydroxyethyl methacrylate) nanocomposites for colon cancer drug delivery, *Appl. Clay Sci.* 97–98 (2014) 33–42. doi:10.1016/j.clay.2014.06.002.
- [15] H. Hemmatpour, V. Haddadi-Asl, H. Roghani-Mamaqani, Synthesis of pH-sensitive poly (N,N-dimethylaminoethyl methacrylate)-grafted halloysite nanotubes for adsorption and controlled release of DPH and DS drugs, *Polymer (Guildf)*. 65 (2015) 143–153. doi:10.1016/j.polymer.2015.03.067.
- [16] S. Riela, M. Massaro, C.G. Colletti, A. Bommarito, C. Giordano, S. Milioto, R. Noto, P. Poma, G. Lazzara, Development and characterization of co-loaded curcumin/triazole-halloysite systems and evaluation of their potential anticancer activity, *Int. J. Pharm.* 475 (2014) 613–623. doi:10.1016/j.ijpharm.2014.09.019.
- [17] F. Jamshidzadeh, A. Mohebbi, M. Abdouss, Three-ply biocompatible pH-responsive nanocarriers based on HNT sandwiched by chitosan / pectin layers for controlled release of phenytoin sodium, *Int. J. Biol. Macromol.* 150 (2020) 336–343. doi:10.1016/j.ijbiomac.2020.02.029.
- [18] M. Massaro, R. Amorati, G. Cavallaro, S. Guernelli, G. Lazzara, S. Milioto, R. Noto, P. Poma, S. Riela, Direct chemical grafted curcumin on halloysite nanotubes as dual-responsive prodrug for pharmacological applications, *Colloids Surfaces B Biointerfaces*. 140 (2016) 505–513. doi:10.1016/j.colsurfb.2016.01.025.
- [19] E. Licandro, C. Rigamonti, M. Ticozzelli, M. Monteforte, C. Baldoli, C. Giannini, S. Maiorana, Synthesis and Functionalization of Novel Tetrathia[7]helicenes as New Push-Pull Systems, *Synthesis (Stuttg)*. 2006 (2006) 3670–3678. doi:10.1055/s-2006-950222.
- [20] Y. Shen, C.-F. Chen, Helicenes: Synthesis and Applications, *Chem. Rev.* 112 (2012) 1463–1535. doi:10.1021/cr200087r.
- [21] G. Song, J. Ren, Recognition and regulation of unique nucleic acid structures by small molecules, *Chem. Commun.* 46 (2010) 7283. doi:10.1039/c0cc01312a.
- [22] Y. Xu, Y.X. Zhang, H. Sugiyama, T. Umamo, H. Osuga, K. Tanaka, (P)-helicene displays chiral selection in binding to Z-DNA, *J. Am. Chem. Soc.* 126 (2004) 6566–6567. doi:10.1021/ja0499748.
- [23] S. Sakunkaewkasem, A. Petdum, W. Panchan, J. Sirirak, A. Charoenpanich, T. Sooksimuang, N. Wanichacheva, Dual-Analyte Fluorescent Sensor Based on [5]Helicene Derivative with Super Large Stokes Shift for the Selective Determinations of Cu²⁺ or Zn²⁺ in Buffer Solutions and Its Application in a Living Cell, *ACS Sensors*. 3 (2018) 1016–1023. doi:10.1021/acssensors.8b00158.
- [24] K. Shinohara, Y. Sannohe, S. Kaieda, K. Tanaka, H. Osuga, H. Tahara, Y. Xu, T. Kawase, T. Bando, H. Sugiyama, A Chiral Wedge Molecule Inhibits Telomerase Activity, *J. Am. Chem. Soc.* 132 (2010) 3778–3782. doi:10.1021/ja908897j.

- [25] Z. An, M. Yamaguchi, Chiral recognition in aggregation of gold nanoparticles grafted with helicenes, *Chem. Commun.* 48 (2012) 7383. doi:10.1039/c2cc32735j.
- [26] D. Balogh, Z. Zhang, A. Ceconello, J. Vavra, L. Severa, F. Teply, I. Willner, Helquat-Induced Chiroselective Aggregation of Au NPs, *Nano Lett.* 12 (2012) 5835–5839. doi:10.1021/nl303179s.
- [27] S. Cauteruccio, C. Bartoli, C. Carrara, D. Dova, C. Errico, G. Ciampi, D. Dinucci, E. Licandro, F. Chiellini, A nanostructured PLGA system for cell delivery of a tetrathiahelicene as a model for helical DNA intercalators, *Chempluschem.* 80 (2015) 490–493. doi:10.1002/cplu.201402347.
- [28] M. Li, L.-H. Feng, H.-Y. Lu, S. Wang, C.-F. Chen, Tetrahydro[5]helicene-Based Nanoparticles for Structure-Dependent Cell Fluorescent Imaging, *Adv. Funct. Mater.* 24 (2014) 4405–4412. doi:10.1002/adfm.201400199.
- [29] A. Babič, S. Pascal, R. Duwald, D. Moreau, J. Lacour, E. Allémann, [4]Helicene-Squalene Fluorescent Nanoassemblies for Specific Targeting of Mitochondria in Live-Cell Imaging, *Adv. Funct. Mater.* 27 (2017) 1701839. doi:10.1002/adfm.201701839.
- [30] S. Maiorana, A. Papagni, E. Licandro, R. Annunziata, P. Paravidino, D. Perdicchia, C. Giannini, M. Bencini, K. Clays, A. Persoons, A convenient procedure for the synthesis of tetrathia-[7]-helicene and the selective α -functionalisation of terminal thiophene ring, *Tetrahedron.* 59 (2003) 6481–6488. doi:10.1016/S0040-4020(03)01056-1.
- [31] E. Joussein, S. Petit, J. Churchman, B. Theng, D. Righi, B. Delvaux, Halloysite clay minerals — a review, *Clay Miner.* 40 (2005) 383–426. doi:10.1180/0009855054040180.
- [32] D. Meroni, L. Lo Presti, G. Di Liberto, M. Ceotto, R.G. Acres, K.C. Prince, R. Bellani, G. Soliveri, S. Ardizzone, A Close Look at the Structure of the TiO₂-APTES Interface in Hybrid Nanomaterials and Its Degradation Pathway: An Experimental and Theoretical Study, *J. Phys. Chem. C.* 121 (2017) 430–440. doi:10.1021/acs.jpcc.6b10720.
- [33] G. Soliveri, V. Pifferi, R. Annunziata, L. Rimoldi, V. Aina, G. Cerrato, L. Falciola, G. Cappelletti, D. Meroni, Alkylsilane-SiO₂ Hybrids. A Concerted Picture of Temperature Effects in Vapor Phase Functionalization, *J. Phys. Chem. C.* 119 (2015) 15390–15400. doi:10.1021/acs.jpcc.5b04048.
- [34] P.M. Dietrich, C. Streeck, S. Glamsch, C. Ehlert, A. Lippitz, A. Nutsch, N. Kulak, B. Beckhoff, W.E.S. Unger, Quantification of Silane Molecules on Oxidized Silicon: Are there Options for a Traceable and Absolute Determination?, *Anal. Chem.* 87 (2015) 10117–10124. doi:10.1021/acs.analchem.5b02846.
- [35] N. Rathor, S. Panda, Aminosilane densities on nanotextured silicon, *Mater. Sci. Eng. C.* 29 (2009) 2340–2345. doi:10.1016/j.msec.2009.06.003.
- [36] A. Bossi, L. Falciola, C. Graiff, S. Maiorana, C. Rigamonti, A. Tiripicchio, E. Licandro, P.R. Mussini, Electrochemical activity of thiahelicenes: Structure effects and electrooligomerization ability, *Electrochim. Acta.* 54 (2009) 5083–5097. doi:10.1016/j.electacta.2009.02.026.
- [37] Y. Zhang, X. He, J. Ouyang, H. Yang, Palladium nanoparticles deposited on silanized halloysite nanotubes: synthesis, characterization and enhanced catalytic property, *Sci. Rep.* 3 (2013) 2948. doi:10.1038/srep02948.

- [38] K.M. Ng, Y.T.R. Lau, C.M. Chan, L.T. Weng, J. Wu, Surface studies of halloysite nanotubes by XPS and ToF-SIMS, *Surf. Interface Anal.* 43 (2011) 795–802. doi:10.1002/sia.3627.
- [39] R.G. Acres, A. V. Ellis, J. Alvino, C.E. Lenahan, D.A. Khodakov, G.F. Metha, G.G. Andersson, Molecular Structure of 3-Aminopropyltriethoxysilane Layers Formed on Silanol-Terminated Silicon Surfaces, *J. Phys. Chem. C.* 116 (2012) 6289–6297. doi:10.1021/jp212056s.
- [40] Halloysite dataset, (n.d.). <http://rruff.geo.arizona.edu/AMS/result.php?mineral=Halloysite> (accessed October 4, 2019).
- [41] J. Noh, E. Ito, K. Nakajima, J. Kim, H. Lee, M. Hara, High-Resolution STM and XPS Studies of Thiophene Self-Assembled Monolayers on Au(111), *J. Phys. Chem. B.* 106 (2002) 7139–7141. doi:10.1021/jp020482w.
- [42] M. Kehrre, J. Duchoslav, A. Hinterreiter, M. Cobet, A. Mehic, T. Stehrer, D. Stifter, XPS investigation on the reactivity of surface imine groups with TFAA, *Plasma Process. Polym.* 16 (2019) 1800160. doi:10.1002/ppap.201800160.
- [43] N. Graf, E. Yegen, T. Gross, A. Lippitz, W. Weigel, S. Krakert, A. Terfort, W.E.S. Unger, XPS and NEXAFS studies of aliphatic and aromatic amine species on functionalized surfaces, *Surf. Sci.* 603 (2009) 2849–2860. doi:10.1016/j.susc.2009.07.029.
- [44] G.M. Su, S.N. Patel, C.D. Pemmaraju, D. Prendergast, M.L. Chabinyc, First-Principles Predictions of Near-Edge X-ray Absorption Fine Structure Spectra of Semiconducting Polymers, *J. Phys. Chem. C.* 121 (2017) 9142–9152. doi:10.1021/acs.jpcc.7b01353.
- [45] Q. Peng, K. Efimenko, J. Genzer, G.N. Parsons, Oligomer orientation in vapor-molecular-layer-deposited alkyl-aromatic polyamide films, *Langmuir.* 28 (2012) 10464–10470. doi:10.1021/la3017936.
- [46] M. Massaro, S. Riela, C. Baiamonte, J.L.J. Blanco, C. Giordano, P. Lo Meo, S. Milioto, R. Noto, F. Parisi, G. Pizzolanti, G. Lazzara, Dual drug-loaded halloysite hybrid-based glycocluster for sustained release of hydrophobic molecules, *RSC Adv.* 6 (2016) 87935–87944. doi:10.1039/C6RA14657K.
- [47] P.L. Ritger, N.A. Peppas, A simple equation for description of solute release I. Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or disks., *J. Control. Release.* 5 (1987) 23–36.

CHAPTER 3

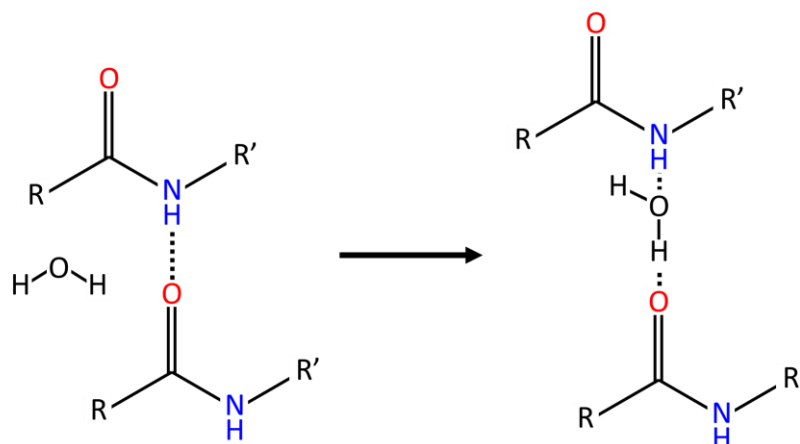
Composites of PA6 and halloysite nanotubes with improved hydrothermal ageing resistance: role of filler physicochemical properties, functionalization and dispersion technique

As previously mentioned, halloysite can be useful as a filler for polymer composites with enhanced properties. The use of fillers for the enhancement of polymer properties has been widely explored in the last decades, as the demand for innovative plastic materials increased. At first, fillers were adopted simply to increase the mass of the product, reducing the amount of expensive polymers. As time passed, however, the role of fillers became more and more relevant, turning them from a cheap production escamotage to integral components for many applications, especially reinforcing the thermomechanical properties of the polymer. Traditional fillers included talc, glass fibres, calcium carbonate and carbon black, but these materials required high loading for a slight improvement in the properties of the composite, causing issues during processing as the filled materials had high viscosity. With the years, these fillers were gradually replaced with nanosized materials, which extremely high specific surface area exponentially multiplied the interface with the polymer matrix, resulting in much greater improvements with much less filler: a 3-5% of nanofiller load achieves the same reinforcing effect of a 20-30% load of micro-sized material. Thus, nanocomposites have emerged as new promising materials, where filler loadings can be kept low without sacrificing the final quality of the product[1,2].

In this context, my investigation focused on the role of surface features of HNT and of their surface modification in promoting the filler compatibility with the chosen polymer matrix, polyamide 6 (PA6).

In the last decades, PA6 has received considerable attention[3] for a broad range of applications including the automotive sector[4], textile industry[5], but also as scaffold for tissue engineering[6] and food packaging[7], owing to its good mechanical performances and high thermal resistance, which are the result of the strong hydrogen bonds in its chemical structure. However, in demanding environmental conditions (*i.e.* extremely high/low temperatures, high humidity, solar exposure and salts absorption), different degradation mechanisms occur and decrease PA6 material performance and durability[8–10]. In particular, in humid conditions, water can interfere with pre-existent hydrogen bonds formed between $-NH$ and $C=O$ groups of neighbouring PA6 chains, as shown in Scheme 3.1, leading to a loss in mechanical cohesion[11–14]. Because the prediction and extension of PA6 durability is a very important issue for

chemists, engineers and final users, numerous studies have been devoted to investigating its hydrothermal ageing.



Scheme 3.1 - Mechanism of the intercalation of polyamide chains with moisture water.

In this context, PA6 properties can be preserved or modified to suit engineering requirements for several applications, using fillers and fibre additives such as silicate clays[15], glass fibre[16], black carbon[17], graphite[18], silica[19] and graphene[20]. However, it should be underlined that in some composites, such as PA6 and glass fibres-based materials, the additive/matrix interface is considered a privileged site for water accumulation during diffusion processes. Since this behaviour can become catastrophic as the additive content increases[21,22], the use of low filler amounts represents a preferable strategy.

By using fillers with nanotubular shape, significant improvements in the composite properties can be obtained using very low loadings, even down to a range of 1-5% w/w, maintaining in this way processing conditions similar to the ones of the neat material[15,23,24]. This is because their high surface/volume ratio favours their dispersion into the polymer matrix. So far, numerous investigations have focused on PA6 functionalization with carbon nanotubes (CNTs)[25–27] and boron nitride nanotubes (BNNTs)[28,29], often with very high loadings (up to 40% w/w[30,31]), due to the interesting mechanical properties of the resulting nanocomposites: carbon-based nanofillers such as CNTs display excellent properties due to their high mechanical strength and high aspect ratio. However, both of these additives are technologically demanding to produce in bulk and functionalize, making them quite expensive and suitable only for limited high added value applications, *e.g.* robotics and organic electronics[32,33]. Thus, there is still a need to develop new types of high-performance PA6-nanocomposites that can be prepared via large scale, low cost fabrication routes.

In this framework, halloysite nanotubes represent a novel 1D natural nanomaterial, morphologically similar to CNTs and BNNTs, with a unique combination of tubular nanostructure, natural availability, low cost, ease of functionalization, good biocompatibility, light colour and high mechanical strength[34–36]. These features can

impart useful mechanical, thermal, and biological properties to HNT-polymer nanocomposites, while keeping costs low[37,38]. Nanocomposites of polyamides and HNT have been reported in the literature to exhibit higher thermal stability[39] and flame inhibition properties[40], as well as enhanced strength without loss of ductility[41], even at very low filler content ($< 5\%$)[42–44]. However, to the author's best knowledge, no previous reports about the effect of HNT filler on the durability of polyamide materials in hydrothermal conditions can be found in the literature, despite the importance of such tests for the assessment of the resistance to environmental conditions and durability of the composites[11–14]. The present study aims at shedding light on this aspect by exploiting the effects of low content (1 and 4% w/w) of HNT fillers on the hydrothermal ageing of PA6 nanocomposites.

As halloysite is a natural mineral, it shows a large morphological variance (from dimension to specific surface area and aspect ratio) depending on the extraction site. This aspect can have a great impact on the final properties of nanocomposites, but it has been often overlooked in previous literature reports. In this regard, here two different sources of HNT with several structural, morphological, optical and surface differences were thoroughly compared.

Moreover, a comparison was made between the two most used filler incorporation strategies for PA6: *in situ* polymerization and melt blending processes. *In situ* polymerization involves the dispersion of modifiers into the monomer followed by polymerization, whilst melt extrusion proceeds via a mechanical blending of filler in the molten polymer. The melt blending approach is more cost effective and straightforward than the *in situ* polymerization technique, but full filler intercalation is not always achievable[45]. On the other side, relatively little work has been reported on the *in situ* polymerization technique, particularly concerning the relationship between the filler dispersion efficiency and PA6-based composites resultant macromolecular properties[45], and no previous work can be found relative to HNT-polyamide composites using this incorporation strategy.

One of the main advantages of HNT is their ease of functionalization, which enables the tailoring of their surface properties. Guo et al.[39] reported the covalent incorporation of HNT functionalized with 3-(trimethoxysilyl)propyl methacrylate within a PA6 matrix, showing good incorporation and enhanced thermal and mechanical properties of the resulting composite. Inspired by this example, in the present work, both HNT fillers in their pristine state and after functionalization with a different silane molecule, (3-aminopropyl)triethoxysilane (APTES), were compared; this surface modification was adopted to increase compatibility between the filler surface and the organic matrix, thanks to the presence of the APTES amine group, as well as to reduce the surface hydrophilicity.

For all of the prepared composites, the effect of HNT incorporation on the composite molecular weights, molecular weight distribution, thermal and rheological properties, together with the filler dispersion state inside the polymer matrix, was investigated with respect to standard PA6. In particular, the comparison between *in situ* and melt blending methods has highlighted the role of HNT surface charge with respect to the filler dispersion efficiency. Finally, the composite resistance to humidity was determined via accelerated ageing tests. It is well known that the macroscopic properties of most

polyamides are affected by hydrothermal ageing after few months, or even weeks[46–48]. In the present case, notable differences between the neat PA6 and the composite materials are observed after already 70 days.

3.1 Experimental procedures

3.1.1 Materials

ϵ -Caprolactam (99%), 6-aminocaproic acid ($\geq 99\%$), distilled water Chromasolv® ($\geq 99.9\%$), toluene (anhydrous, $\geq 99.8\%$), potassium nitrate (KNO_3), 2-propanol, (3-aminopropyl)triethoxysilane (APTES, $\geq 98\%$) and dichloromethane (DCM anhydrous, $\geq 99.8\%$) were supplied by Sigma Aldrich and used without further purification. The commercial polyamide 6 “Technyl® C 246 natural” (PA6TECH) was purchased from Solvay Chimica Italia S.p.A. Two different kinds of HNT nano-clays were used to prepare nanocomposites: HNT_H was supplied by iMineral Inc. (commercially known as Ultra HalloPure®) and HNT_S was received from Sigma Aldrich.

3.1.2 HNT functionalization

2 g of HNT was suspended in 100 mL of toluene. Then, 1.4 g (6.3 mmol) of APTES was added and the suspension was refluxed under stirring for 7 h. Heating was stopped during the night (15 h) and the retrieved powder was washed with toluene by centrifugation/resuspension cycles (5x40 mL). The solid was then dried in the oven at 90°C for 24 h.

3.1.3 HNT characterization

The specific surface area (SSA) of the samples was measured through N_2 adsorption-desorption isotherms in subcritical conditions, utilizing a Coulter SA 3100 apparatus, analyzed according to the Brunauer Emmett Teller (BET) method.

X-ray diffraction (XRD) patterns of the samples were acquired using a Siemens D5000 diffractometer equipped with a $\text{Cu K}\alpha$ source ($\lambda = 0.15406 \text{ nm}$), working at 40 kV x 40 mA nominal X-ray power, at room temperature. $\theta : 2\theta$ scans were performed between 10° and 80° with a step of 0.02° .

The oxide ζ -potential was measured using a Zetasizer Nano ZS (Malvern Instruments), operating at $\lambda = 633 \text{ nm}$ with a solid state He-Ne laser at a scattering angle of 173° , using the dip-cell kit. Powders were suspended in a 0.01 M KNO_3 aqueous solution (0.5 mg mL^{-1}). Each ζ -potential value was averaged from at least three measurements.

Thermogravimetric analyses (TGA) were performed on bare and modified HNT using a Mettler-Toledo TGA/DSC 3+ STAR System, between 30 and 900 °C with a rate of 3 °C/min under air flux.

Transmission electron microscopy (TEM) images were obtained with a Zeiss LEO 912ab Energy Filtering TEM, equipped with a CCD-BM/1K system, operating at an acceleration voltage of 120 kV. Samples were suspended in 2-propanol or water (1 mg/mL) and deposited on Cu holey carbon grids (200 mesh).

Fourier transform infrared (FTIR) spectra were acquired using a Perkin Elmer Spectrum 100 spectrophotometer in Attenuated Total Reflectance (ATR) mode, registering 12 scans between 4000 and 400 cm^{-1} with a resolution of 4.0 cm^{-1} . A single-bounce diamond crystal was used with an incidence angle of 45°.

Diffuse reflectance spectra (DRS) were collected using a Shimadzu UV2600 spectrophotometer.

3.1.4 PA6 and HNT nanocomposites (PA6_HNT) preparation and characterization

PA6-HNT composite preparations were carried out by Dr V. Sabatini at the Università degli Studi di Milano, who carried out also some of the characterizations on the composites.

3.1.4.1 In situ polymerization

A 250 cm^3 one-necked round bottom flask was loaded with ϵ -caprolactam (50.0 g), 6-aminocaproic acid (2.5 g) and the selected amount of HNT (either 1 or 4% w/w), and equipped with a polymerization candelabrum having nitrogen inlet and outlet adapters and an overhead mechanical stirrer. The flask was flushed with nitrogen, heated at 270 °C in a polymerization oven and then stirred (150-180 rpm) for 6 h. Afterwards, the reaction mixture was gradually brought back to room temperature. Flowing nitrogen atmosphere was maintained throughout the polymerization reaction. The resulting product was washed from unreacted ϵ -caprolactam with boiling water for 24 h, and dried in vacuum (about 4 mbar) at 100 °C for at least 12 h. For the sake comparison, neat PA6 was synthesized using the same conditions.

3.1.4.2 Melt processing

Melt blended PA6-HNT nanocomposites (with filler content 1 or 4% w/w) were prepared using a ThermoScientific HAAKE Process 11 co-rotating twin screw extruder, with extrusion temperatures set at 235, 235, 235, 235, 235, 230, 217 and 210 °C from hopper to die respectively, a screw speed of 160 rpm, and a feed rate of 3 rpm. The extruded strands were quenched in water, air dried and then pelletized. Neat PA6, used as reference, was prepared by extruding PA6TECH under the same processing conditions. Prior to the use, nano-clays and PA6TECH pellets were dried under nitrogen atmosphere for 12 h at 60°C in an oven to remove physisorbed water.

3.1.4.3 PA6 nanocomposites characterization

The HNT actual content in PA6-nanocomposites was determined via dynamic TGA analyses performed from 30 to 900°C at 10°C/min, under nitrogen flow, using a Perkin Elmer TGA 4000 Instrument.

Scanning Electron Microscopy (SEM) studies of PA6 nanocomposites fracture surfaces were carried out on a Leica Electron Optics 435 VP microscope, working at an acceleration voltage of 15 kV and 50 pA of current probe and equipped with an energy dispersive X-ray (EDX) spectrometer (Philips, XL-30W/TMP), to investigate the HNT dispersion in PA6 polymer matrix. The samples were manually fractured after cooling

in liquid nitrogen and sputter-coated with a 20 nm thick gold layer in rarefied argon, using an Emitech K550 Sputter Coater, with a current of 20 mA for 180 s.

The isothermal crystallization behaviour of PA6 and its nanocomposites was determined using a Mettler Toledo DSC1 calorimeter; analyses were conducted under nitrogen atmosphere, weighting 5-10 mg of each sample in a standard 40 μ L aluminium crucible and using an empty 40 μ L crucible as reference.

In addition, non-isothermal behaviour was studied using the following program: i) heating at a rate of 10°C/min from 25 to 250 °C; ii) 5 min of isotherm at 250 °C; iii) cooling from 250 to 25 °C at 10 °C/min (crystallization temperature, T_c and crystallization enthalpy, ΔH_c , were determined during cooling); iv) 5 min of isotherm at 25 °C and v) heating from 25 to 250 °C at 10 °C/min (melting temperature, T_m , and heat of fusion, ΔH_f , were measured during heating). ΔH_f was used to calculate the level of crystallinity, defined by the ratio of ΔH_f to the heat of fusion of the purely crystalline forms of PA6 (ΔH_f^0). Since ΔH_f^0 values of the α and γ crystalline forms of PA6 are nearly identical, the level of crystallinity was calculated using the average of the two *i.e.* 240 J/g[35].

Rheological analyses, conducted using frequency sweep experiments, were performed with a Physica MCR 300 rotational rheometer with a parallel plate geometry (ϕ = 25 mm; 1 mm distance between plates). Neat PA6 and PA6-HNT nanocomposites were dried in a vacuum oven (around 4 mbar) at 100 °C for 12 h and linear viscoelastic regimes were studied. Strain was set equal to 5% and curves of complex viscosity as function of frequency were recorded, taking 30 points ranging from 100 Hz to 0.1 Hz with a logarithmic progression, at 250 °C.

The molecular weight properties were evaluated using a Size Exclusion Chromatography (SEC) system having a Waters 1515 Isocratic HPLC pump, four Waters Styragel (103Å-104Å-105Å-500Å) columns and a UV detector (Waters 2487 Dual λ Absorbance Detector) at 244 nm, using a flow rate of 1 cm³ min⁻¹ and 15 μ L as injection volume. Samples were prepared dissolving 10-15 mg of polymer in 1 cm³ of anhydrous DCM after N-trifluoroacetylation[49]; before the analysis, the solution was filtered with 0.45 μ m filters. Molecular weight data were expressed in polystyrene (PS) equivalents. The calibration was built using monodispersed PS standards having the following nominal peak molecular weight (M_p) and molecular weight distribution (D): M_p = 1600 kDa (D $\leq$ 1.13), M_p = 1150 kDa (D $\leq$ 1.09), M_p = 900 kDa (D $\leq$ 1.06), M_p = 400 kDa (D $\leq$ 1.06), M_p = 200 kDa (D $\leq$ 1.05), M_p = 90 kDa (D $\leq$ 1.04), M_p = 50400 Da (D = 1.03), M_p = 37000 Da (D = 1.02), M_p = 17800 Da (D = 1.03), M_p = 6520 Da (D = 1.03), M_p = 5460 Da (D = 1.03), M_p = 2950 Da (D = 1.06), M_p = 2032 Da (D = 1.06), M_p = 1241 Da (D = 1.07), M_p = 906 Da (D = 1.12); ethyl benzene (molecular weight = 106 g/mol). For all analyses, 1,2-dichlorobenzene was used as internal reference.

3.1.5 Hydrothermal ageing tests

Samples for the accelerated ageing test were prepared via injection moulding with a BABYPLAST 610P instrument and were immersed in distilled water at a set temperature of 90°C for 70 days in glass jar containers. The main parameters of the injection moulding are reported in Table 3.1. The ageing temperature was chosen to accelerate the diffusion process as well as to accentuate the materials degradation mechanism[12,50]. Samples were fully immersed and periodically, *i.e.* after 25 (600 h), 50 (1200 h) and 70 days (1680 h), weighted using a digital balance of 0.0001 g accuracy. The moisture uptake (M_t) was measured through a gravimetric method, as reported in Equation 1.

$$M_t (\%) = (m_t - m_o)/m_o \times 100 \quad (1)$$

where “ m_t ” and “ m_o ” are the wet and dry weights of the sample after any specific time t . Aged samples were analyzed via rheological, SEC and DSC analyses, according to the experimental procedures described in paragraph 2.3.3.

Parameter	Operative data
Sample temperature	255-275 °C
Mould temperature	80 °C
Injection volume	44 cm ³
Injection pressure	1500 bar
Injection rate	40 cm ³ /s
Holding pressure	400 bar
Holding time	30 s
Cooling time	30 s

Table 3.1 - Injection moulding parameters for PA6 nanocomposites specimen preparation.

3.2 Results and discussion

3.2.1 HNT characterization and functionalization

As previously stated, HNT_H and HNT_S fillers were acquired from different suppliers, and in order to investigate the differences between the two types of nanotubes, they were analyzed via BET, XRD, TGA and TEM. The two powders present different colours, HNT_S being white while HNT_H is brownish, probably due to iron impurities (Figure 3.1). As reported in Table 1, HNT_S powder possesses a specific surface area of 53 m²/g, in good agreement with literature data[51,52]; on the other hand, HNT_H sample shows a lower surface area of 34 m²/g. According to XRD patterns reported in Figure 3.2 A, HNT_S filler presents several impurity phases alongside halloysite, including kaolinite, quartz and gibbsite. These findings are in good agreement with previous literature reports on HNT samples from the same supplier[51,53]. Instead, HNT_H powder appears purer, as apparent from the absence or much lower intensity of impurity peaks, and more crystalline, as shown by the sharper halloysite (10 Å) peaks. Our

diffractogram is in good agreement with data from the supplier, showing only minor crystalline impurities, such as quartz (<1%) and kaolinite (<10%).



Figure 3.1 - Visual comparison of HNT_s (A) and HNT_H (B) and their respective composites prepared via injection moulding: PA6_{melt}_HNT_s_4 (C) and PA6_{melt}_HNT_H_4 (D).

TGA measurements are reported in Figure 3.2 B. The initial mass loss, between 50 and 300 °C can be associated with the removal of physisorbed and interlayer water, in this order. The most severe weight loss, at around 500 °C, can be attributed to the dehydroxylation of the inner surface[52]. The two sources of HNT show a slight difference in weight loss at lower temperatures. This can be related to the amount of physisorbed water on their surface: as HNT_s sample has a higher surface area (Table 3.2), it also carries more water, resulting in a greater weight loss. Moreover, upon APTES addition a weight loss at around 250 °C can be appreciated, related to the degradation of the aminopropyl chain. Overall, in both cases loading is around 5% (Table 3.2), notwithstanding HNT_H lower surface area. This suggests that in this case functionalization was more efficient.

TEM images of untreated HNT were acquired to determine the difference in morphology between the two sources (Figure 3.2 C). As can be seen in Table 3.2, the two families of HNT show greatly different aspect ratios: HNT_H appear much more elongated than HNT_s, as their aspect ratio is three times larger, and also much less fragmented.

FTIR spectra of HNT samples are reported in Figure 3.2 D, in the 1800-1200 cm⁻¹ range. As can be seen, HNT_s filler shows a more intense water bending peak at 1650 cm⁻¹, in line with what found by TGA. After the functionalization with APTES, few signals between 1600 and 1300 cm⁻¹ become appreciable: the peak at 1561 cm⁻¹ can be attributed to -NH₂ scissoring modes of APTES amino groups, while that at 1490 cm⁻¹

can be related to the symmetric deformation of $-\text{NH}_3^+$ moieties. Finally, the band at 1335 cm^{-1} can be assigned to C-N stretching modes[54].

Interestingly, one of the most dramatic differences between the two sources of HNT resides in their surface charge: as reported in Table 3.2, the recorded ζ -potential at spontaneous pH for HNT_H is much more negative than that of HNT_s and this difference persists even after surface functionalization with APTES even though, due to its positively charged $-\text{NH}_3^+$ groups, it should raise the value of surface charge.

Sample	SSA (m^2/g)	Avg. length (nm)	Avg. aspect ratio	ζ -potential (bare HNT) (mV)	ζ -potential (HNT_APTES) (mV)	APTES loading (%)
HNT_s	53	400 ± 300	5 ± 3	-31 ± 2	$+5.9 \pm 0.6$	4.6
HNT_H	34	1100 ± 800	15 ± 9	-59 ± 3	-44 ± 2	5.1

Table 3.2 - Physicochemical properties of bare and functionalized HNT: specific surface area (SSA), average length and aspect ratio, ζ -potential at spontaneous pH, and APTES loading from TGA.

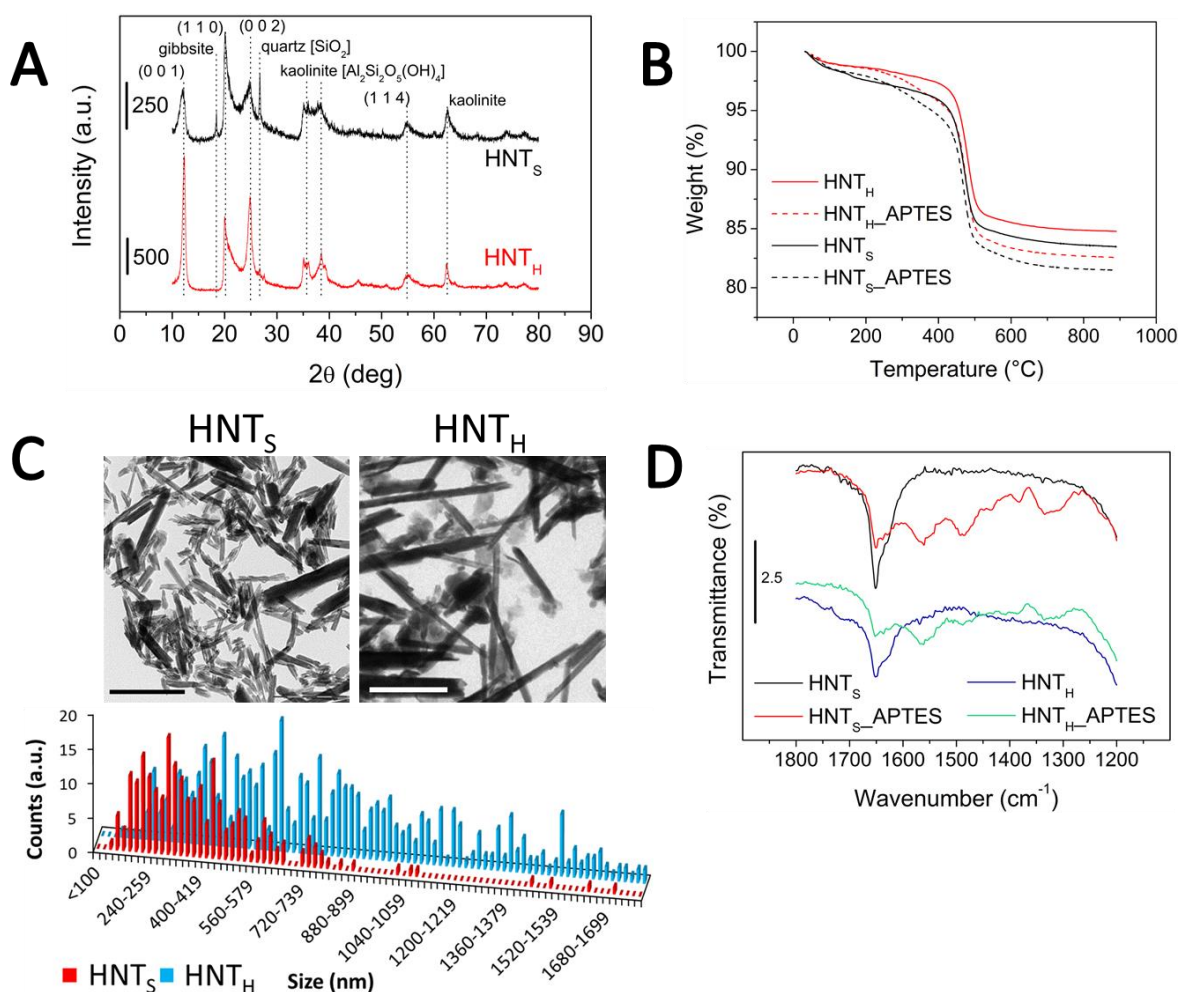


Figure 3.2 - Characterization of adopted HNT powders: A) XRD patterns with indexing of the halloysite peaks and main impurities; B) TGA curves; C) TEM images (scale bar: 1 μm) and D) FTIR spectra of bare and APTES-modified fillers.

3.2.2 Synthesis of PA6 and PA6-HNT nanocomposites via *in situ* polymerization and melt blending processes

The dispersion of HNT_H and HNT_s inside PA6 polymer matrix was performed comparing two different incorporation routes, *i.e.* *in situ* polymerization and melt blending processes. The fillers, both unmodified and functionalized with APTES, were tested at 1 and 4% w/w loadings. Table 2 lists all of the prepared samples, along with the actual content of HNT inside the PA6 nanocomposites, as determined via TGA analyses. The latter are, in all cases, very close to the theoretical amount.

Preparation route	Sample	HNT _{theoretical} (% w/w)	HNT _{real} (% w/w)	Avrami data		
				onset T_c (°C)	n	$t_{1/2}$ (s)
In situ polymerization	PA6 _{in situ}	-	-	190	1.6	63
				199	1.8	408
	PA6 _{in situ} _HNT _H _1	1	0.8	190	1.0	135
	PA6 _{in situ} _HNT _S _1	1	0.9	199	2.3	408
	PA6 _{in situ} _HNT _H _4	4	3.3	190	1.1	163
	PA6 _{in situ} _HNT _S _4	4	4.0	199	1.6	408
	PA6 _{in situ} _HNT _H _1_APTES	1	0.7	190	1.0	137
	PA6 _{in situ} _HNT _S _1_APTES	1	1.0	199	2.4	420
	PA6 _{in situ} _HNT _H _4_APTES	4	4.0	190	1.2	174
	PA6 _{in situ} _HNT _S _4_APTES	4	3.9	199	1.6	408
Melt extrusion	PA6 _{melt}	-	-	200	1.8	135
	PA6 _{melt} _HNT _H _1	1	1.0	200	2.3	135
	PA6 _{melt} _HNT _S _1	1	0.6	200	2.6	135
	PA6 _{melt} _HNT _H _4	4	4.0	200	2.3	135
	PA6 _{melt} _HNT _S _4	4	4.0	200	2.1	135
	PA6 _{melt} _HNT _H _1_APTES	1	0.8	200	2.3	126
	PA6 _{melt} _HNT _S _1_APTES	1	0.7	200	2.1	126
	PA6 _{melt} _HNT _H _4_APTES	4	3.8	200	2.0	135
	PA6 _{melt} _HNT _S _4_APTES	4	3.6	200	2.0	135

Table 3.3 - PA6 nanocomposites prepared via in situ polymerization and melt blending processes: theoretical and actual loading from TGA analyses, and kinetic analysis of isothermal crystallization data obtained via Avrami equation.

It should be noted that at the highest filler content (4% w/w) HNT_H noticeably alters the original white colour of neat PA6 (Figure 3.1), as also shown in DRS spectra (Figure 3.3).

To investigate the dispersion efficiency of HNT_H and HNT_S by the two preparation methods, the morphology of cryo-fractured PA6-HNT nanocomposite samples prepared was studied via SEM analyses; the presence of HNT-based aggregates was also investigated via EDX measurements. Representative images of 4% w/w composites are reported in Figure 3.4 and Figure 3.5.

In *in situ* polymerization samples, both unmodified and silane-modified HNT_S form micrometric aggregates (Figure 3.4 C and G red circles), as supported by EDX analyses (data not shown), indicating that the filler has not been efficiently dispersed in the polymer matrix due to its poor interaction with PA6[43]. On the other side, both pristine and functionalized HNT_H fillers are homogeneously dispersed in the polymer and no aggregates are present (Figure 3.4 A and E). The reason behind this different behaviour is probably the greater surface charge of HNT_H, which more effectively separates nanotubes due to same-charge repulsing interactions. This effect, together with the higher aspect ratio leading to a more pronounced self-ordering, might favour a more homogeneous distribution of the filler in PA6_HNT_H composites, whereas HNT_S

aggregates, possibly already present in the untreated powder, remain in the polymer matrix.

The dispersion of HNT_H further improves with APTES functionalization: APTES-modified HNT_H are enclosed and well-penetrated within the fractured PA6 surface. This observation can be explained considering that, while the surface charge remains high upon functionalization (Table 3.2), the amino functional groups of APTES promote the compatibility between the functionalized filler and the PA6 matrix[55].

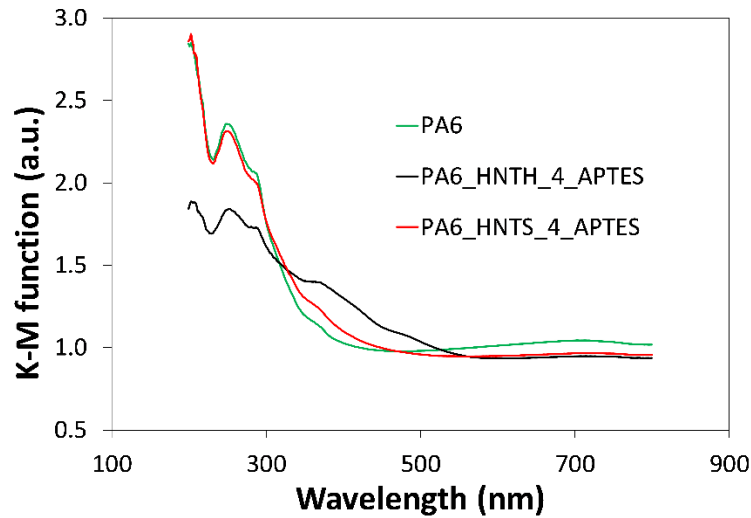


Figure 3.3 - DRS spectra of neat, PA6_HNT_H_4_APTES and PA6_HNT_S_4_APTES nanocomposites prepared via melt blending.

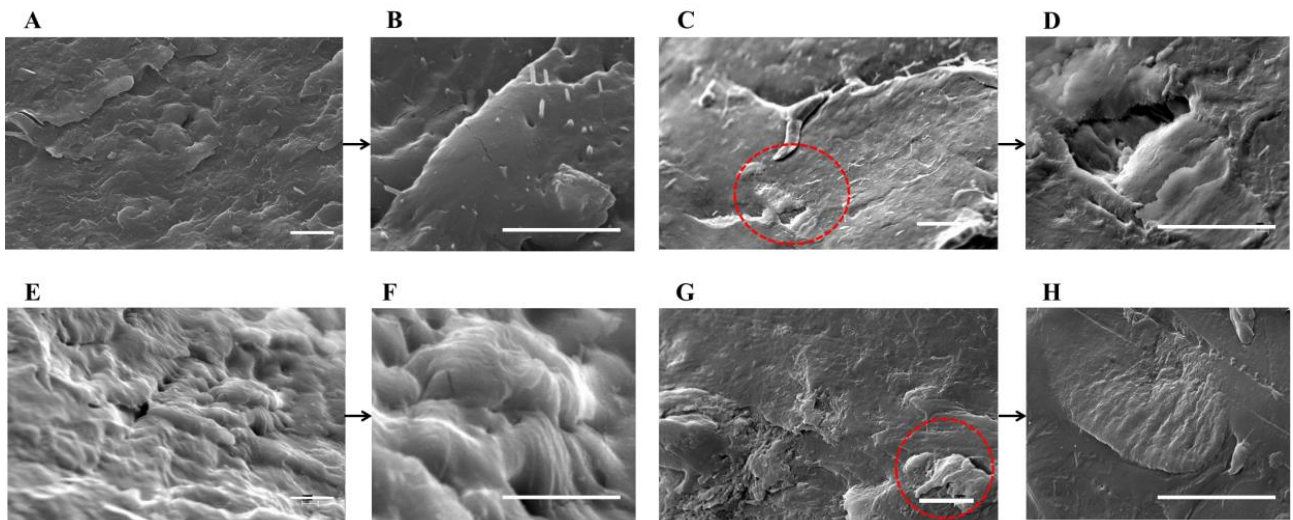


Figure 3.4 - SEM images of A, B) PA6_{in situ}_HNT_H_4, C, D) PA6_{in situ}_HNT_S_4, E, F) PA6_{in situ}_HNT_H_4_APTES and G, H) PA6_{in situ}_HNT_S_4_APTES (scale bar 2 μm).

In the case of nanocomposites prepared via melt extrusion process (Figure 3.5), no significant difference related to the use of HNT_S/HNT_H and APTES is detectable: in

fact, the nanotubes distribution appears to be uniform across all the specimens and the good interaction between HNT and PA6 matrix seems to be independent of surface functionalization. However, it should be noted that in the case of melt blending, the HNT dispersion, although homogeneous, is essentially limited to a superficial layer, as also supported by the performance tests (see Sections 3.2.4 and 3.2.5). On the other hand, the *in situ* polymerization guarantees a bulk penetration of the filler. This difference may be explained by the fact that, as opposed to extrusion, during *in situ* preparation HNT fillers have more time to disperse in the medium, allowing the filler to spread within the polymer bulk.

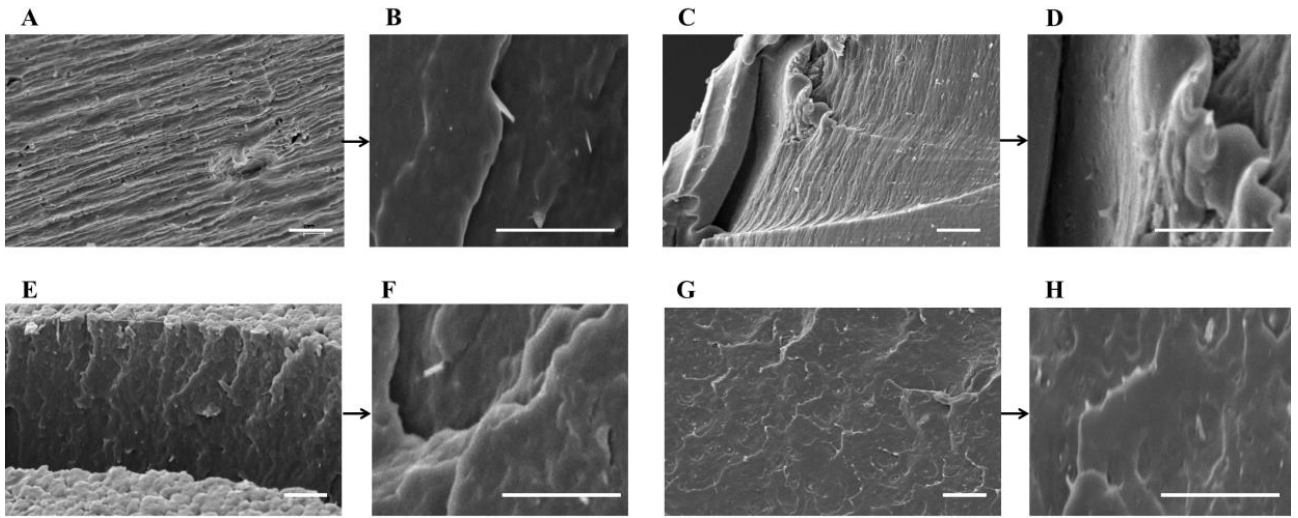


Figure 3.5 - SEM images of A, B) PA6_{melt}_HNT_H_4, C, D) PA6_{melt}_HNT_S_4, E, F) PA6_{melt}_HNT_H_4_APTES and G, H) PA6_{melt}_HNT_S_4_APTES (scale bar: 2 μm).

3.2.3 Isothermal crystallization behaviour of PA6 nanocomposites

The crystallization behaviour of a crystalline polymer can be distinguished in two main processes, nucleation and crystal growth. The initial stage of crystallization, *i.e.* primary crystallization, can be analyzed by the Avrami kinetic equation (Equation 2).

$$1 - X(t) = \exp(-K t^n) \quad (2)$$

where $X(t)$ is the fraction of crystallized polymer at time t , n is the parameter that details nucleation and growth processes, and K is the crystallization rate constant. Linear regression was adopted to obtain n and K from plots of the data in the form of Equation 3.

$$\ln [1 - \ln (1 - X(t))] = \ln K + n \ln t \quad (3)$$

Based on the Avrami theory, n is related to the geometry of the crystal growth and on the type of nucleation.

Here, isothermal crystallization behaviour was studied by heating the sample from 25 to 250 °C at 10°C/min, held for 1 min to ensure melting, then rapidly cooled to i) 199 °C in the case of PA6_HNT_S nanocomposites prepared via *in situ* polymerization, ii)

190 °C for PA6_HNT_H synthesized *in situ* and iii) 200 °C for all samples prepared via melt processing. Neat PA6 was consequently analysed at 190, 199 and 200 °C. The chosen crystallization temperatures (190 / 199 / 200 °C) were determined via a series of experiments conducted at various crystallization temperatures. Below the indicated onset crystallization temperature, the crystallization peak overlapped with the initial transient portion of the heat flow curve. Setting the onset temperature as shown above deletes this problem, while enabling to complete the crystallization process in a reasonable time. According to Fornes et al.[56], the different crystallization behaviour is probably due to the different molecular weight of PA6 in the different composites. Moreover, in the case of PA6_{in situ} nanocomposites, there can be an effect of the different interaction of the two tested HNT with the polymer matrix, as previously discussed. As reported in Table 3.3 (6th column), *n* values for pure PA6_{in situ} and PA6_{melt} samples range from 1.6_{T=190°C} to 1.8_{T=199 and 200°C} and are consistent with values reported in the literature[57]. In PA6_{in situ} nanocomposites, *n* shows a decrease with the addition of HNT_H filler and is not significantly affected by the presence of the functionalizing agent. According to Gurato et al.[58], this behaviour suggests that small amounts of filler (lower than 5-10% w/w) can act as homogeneous nucleation sites followed by mono and bi-dimensional growth. On the other side, HNT_s samples overall show higher *n* values, possibly due to the not satisfactory HNT_s dispersion in PA6 matrix that enhances the formation of heterogeneous nucleation sites[58].

The *n* values for extruded materials are generally higher than the ones obtained by *in situ* polymerization. Also in this case, functionalization with APTES does not significantly change the PA6 nanocomposites crystallization behaviour. As shown by Khanna et al.[59], the increase in *n* can be related to memory effects associated with the processing of PA6. As a matter of fact, numerous studies have shown that the processing history of the polymer, *e.g.* melting, cooling, pelletizing and so on, are often not fully erased when the polymer is annealed at high melting temperatures[56,58,60]. In this way, memory effects associated with thermal and stress histories that remain present in the material after annealing in the melt may also lead to an increased formation of heterogeneous crystallization sites.

The half-time of crystallization (*t*_{1/2}) was calculated according to Equation 4:

$$t_{1/2} = (\ln 2/K)^{1/n} \quad (4)$$

The resulting values for all samples are reported in Table 3.3 (7th column). PA6_{in situ} nanocomposites with bare and APTES-modified HNT_H fillers noticeably decrease their crystallization rate than neat PA6. This effect is due to the good adhesion between PA6 and the filler that impedes the motion of PA6 molecular chains, hampering the crystallization of PA6 matrix[19]. On the other side, pristine and modified HNT_s nanotubes lead a less pronounced decrease of their crystallization rate with respect to neat PA6. Lastly, the crystallization rate of the samples obtained via melt extrusion process is not significantly influenced by the presence of bare and modified HNT.

Overall, the results obtained in the case of samples prepared via melt blending are consistent with the ones reported in the literature[39,56]. The previously discussed inefficient distribution of HNT_s filler inside PA6 matrix by *in situ* polymerization results in a non-homogenous crystallization behaviour of the corresponding composites. Moreover, the different dispersion of HNT_H during *in situ* polymerization and melt

blending process is reflected in the crystallization behaviour of the two types of composite: n and $t_{1/2}$ are lower for the PA6_{in situ} samples, consistently with the better filler incorporation within the bulk polymer matrix.

3.2.4 Non-isothermal crystallization behaviour of PA6 nanocomposites

Non-isothermal crystallization studies were carried out to highlight the effect of pristine and modified fillers onto the crystallization behaviour and crystal structure of the prepared PA6-HNT nanocomposites. Table 3.4 and Table 3.5 report the DSC data of PA6 nanocomposites in non-isothermal heating and cooling conditions.

By comparing the thermal behaviour of each composite with respect to the relative neat PA6, a decrease in T_c (Table 3.4, 2nd column) and ΔH_c (Table 3.5, 2nd column) values is observed upon addition of both pristine and APTES-modified nanotubes by *in situ* polymerization. In particular, T_c values consistently show a decreasing trend at increasing HNT content. As described by Wurm et al.[61], the filler introduction in a polymer matrix hinders its macromolecular chains mobility, thus delays crystal growth. Further evidence of slower kinetics for HNT_H and HNT_S based nanocomposites, can be seen in the χ_c values obtained upon subsequent heating (Table 3.4, 4th column). ΔH_f values used for χ_c calculations are reported in the Supporting Information (Table 3.5, 3rd column). These effects are instead much less appreciable in the case of melt blending composites, in good agreement with isothermal crystallization studies (see Section 3.2) and with the literature[56,62].

Sample	cooling	2 nd heating	
	T_c (°C)	T_m (°C)	X_c (%)
PA6 _{in situ}	188.1	220.8	30.2
PA6 _{in situ} _HNT _H _1	187.2	220.8	23.8
PA6 _{in situ} _HNT _S _1	185.5	220.4	22.3
PA6 _{in situ} _HNT _H _4	182.1	219.0	23.5
PA6 _{in situ} _HNT _S _4	182.2	219.1	22.4
PA6 _{in situ} _HNT _H _1_APTES	187.2	220.3	21.5
PA6 _{in situ} _HNT _S _1_APTES	187.0	220.3	18.3
PA6 _{in situ} _HNT _H _4_APTES	184.6	214.6	14.0
PA6 _{in situ} _HNT _S _4_APTES	185.0	214.4	14.9
PA6 _{melt}	189.4	221.1	24.9
PA6 _{melt} _HNT _H _1	188.8	221.2	20.1
PA6 _{melt} _HNT _S _1	188.9	221.6	24.3
PA6 _{melt} _HNT _H _4	186.2	220.1	17.1
PA6 _{melt} _HNT _S _4	185.9	220.4	21.8
PA6 _{melt} _HNT _H _1_APTES	188.6	221.4	24.4
PA6 _{melt} _HNT _S _1_APTES	188.4	221.4	26.3
PA6 _{melt} _HNT _H _4_APTES	187.1	219.8	21.7
PA6 _{melt} _HNT _S _4_APTES	188.3	219.6	19.3

Table 3.4 - DSC data of PA6-HNT nanocomposites collected during cooling and second heating thermal steps.

Sample	cooling	2 nd heating
	ΔH_c (J/g)	ΔH_f (J/g)
PA6 _{in situ}	73.3	-60.4
PA6 _{in situ} _HNT _H _1	63.1	-57.0
PA6 _{in situ} _HNT _S _1	57.3	-53.6
PA6 _{in situ} _HNT _H _4	61.0	-56.5
PA6 _{in situ} _HNT _S _4	58.5	-53.7
PA6 _{in situ} _HNT _H _1_APTES	54.1	-51.5
PA6 _{in situ} _HNT _S _1_APTES	69.2	-43.8
PA6 _{in situ} _HNT _H _4_APTES	52.9	-33.6
PA6 _{in situ} _HNT _S _4_APTES	58.6	-35.7
PA6 _{melt}	71.7	-59.9
PA6 _{melt} _HNT _H _1	65.1	-48.3
PA6 _{melt} _HNT _S _1	63.5	-58.3
PA6 _{melt} _HNT _H _4	62.2	-41.1
PA6 _{melt} _HNT _S _4	69.7	-52.4
PA6 _{melt} _HNT _H _1_APTES	64.9	-58.5
PA6 _{melt} _HNT _S _1_APTES	59.0	-63.1
PA6 _{melt} _HNT _H _4_APTES	69.5	-52.0
PA6 _{melt} _HNT _S _4_APTES	65.1	-46.4

Table 3.5 - Crystallization enthalpy (ΔH_c) and heat of fusion (ΔH_f) of unaged and aged PA6_HNT nanocomposites collected during cooling and second heating thermal steps.

Furthermore, it is interesting to note that in the case of the samples prepared via *in situ* and melt-blending processes with the 4%w/w of both pristine and modified HNT_H/HNT_S the peak temperature related to the melting transition (T_m) slightly decreases; the above observation may arise from several factors, such as the use of high filler loadings that could disturb the crystalline structures formation[41,63,64], but in particular the formation of a small shoulder prior and close to the endothermic peak, associated with the PA6 γ crystalline form (Figure 3.6), may be a reason[65]. Several works[56,62,66,67] explain that the lower melting point associated with the presence of the γ form could be due to the lower crystalline density and increased melting entropy associated with the reduction in trans-conformation bonding as compared to the α counterpart. On the other hand, the presence of the γ crystalline form and the melting temperature decrease may simply reflect changes and imperfections in crystallites thickness and their distribution. In other words, the lower crystallinity could be due to the inability of polymer chains to be fully incorporated in to growing crystalline lamella; this leads to a smaller crystallite structures and more defects ridden crystalline lamella. Such imperfections in crystalline structure could contribute to the reduction of nanocomposites T_m [68]. Furthermore, several works[39,41,62] report that HNT addition in polyamide-based composites favours polymer polymorphism, *i.e.* the formation of γ crystalline form. In particular, Guo et al.[62] suggested that the Si-O and -OH groups present along HNT surface are capable of forming hydrogen bonds with PA6 amide groups. The interfacial interaction could restrain the mobility of PA6 chains and weaken

the hydrogen bonding interactions between the polymer chains, hindering the formation α -phase crystals.

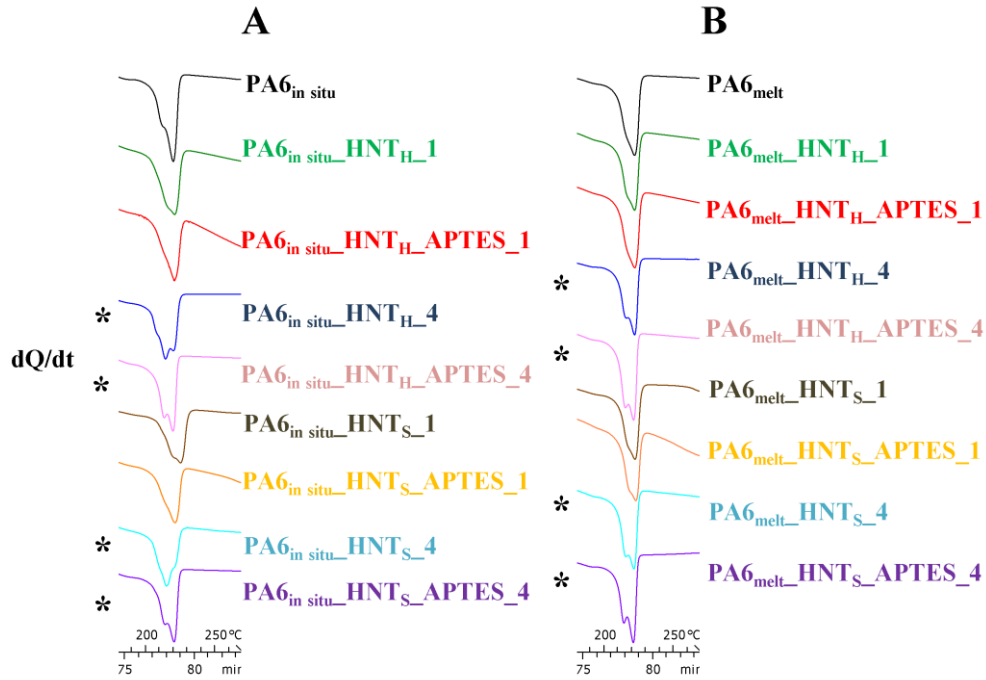


Figure 3.6 - Heating scans of A) PA6_HNT_{in situ} and B) PA6_HNT_{melt} nanocomposites. Sample characterized by the presence of γ form are highlighted with a star (*).

3.2.5 Water uptake and characterization of aged nanocomposites

Figure 3.7 reports the absolute mass variation, calculated with Equation 1, of PA6 and PA6-based nanocomposites conditioned in distilled water at 90°C for 70 days. The ageing temperature of 90°C was chosen, on the hand, because as widely reported in the literature[12,50,69,70], the PA6 hydrothermal stability can be studied at different ageing temperatures, *e.g.* at room temperature or close to water boiling point (~70-90°C), but high ageing temperatures allow to accelerate the same PA6 degradation mechanisms as well as the diffusion processes that occur at lower ageing temperatures. On the other hand, according to Li et al.[71,72] the ageing temperature adopted is higher than the PA6 T_g (~47°C)[56] and therefore allows to accentuate degradation phenomena that otherwise would take weeks or months to happen. The weight of aged samples after 25, 50 and 70 days of accelerated ageing test is reported in Table 3.6. The plotted data show the same trend involving two main steps, corresponding to the ageing periods of 25 and 50-70 days, respectively. In the first 25 days, an initial mass increase is observed as a consequence of a fast initial water uptake, associated with water penetration within PA6 amorphous regions; the mass increase continues until the equilibrium saturation level of the moisture uptake is reached[12]. Then, the equilibrium previously described disappears and a tendency of mass loss is noticed (25-70 days). The latter behaviour can be ascribed to the polymer degradation consequent to hydrolysis phenomena[73].

The moisture uptake differs depending on the tested material. Overall, under the same ageing conditions neat PA6 absorbs more water than modified PA6. Moreover, the

decrease of moisture absorption gets higher with the increase of the modified-filler loading. This latter behaviour is consistent with those reported in previous studies related to the combined use of functionalizing agents and polymers[74,75]. The improved decrease of water uptake in APTES-modified samples can be explained by the fact that amino functional groups present in the polymer matrix decrease PA6-HNT nanocomposites surface free energy[55]. Therefore, the use of modified-HNT fillers hinders and decelerates the mechanism of moisture sorption.

Furthermore, HNT_s seems to be more prone to water uptake than HNT_H; as reported in Section 3.2.1, this behaviour can be related to the HNT_s higher surface area that results in a greater capability of moisture sorption than HNT_H filler.

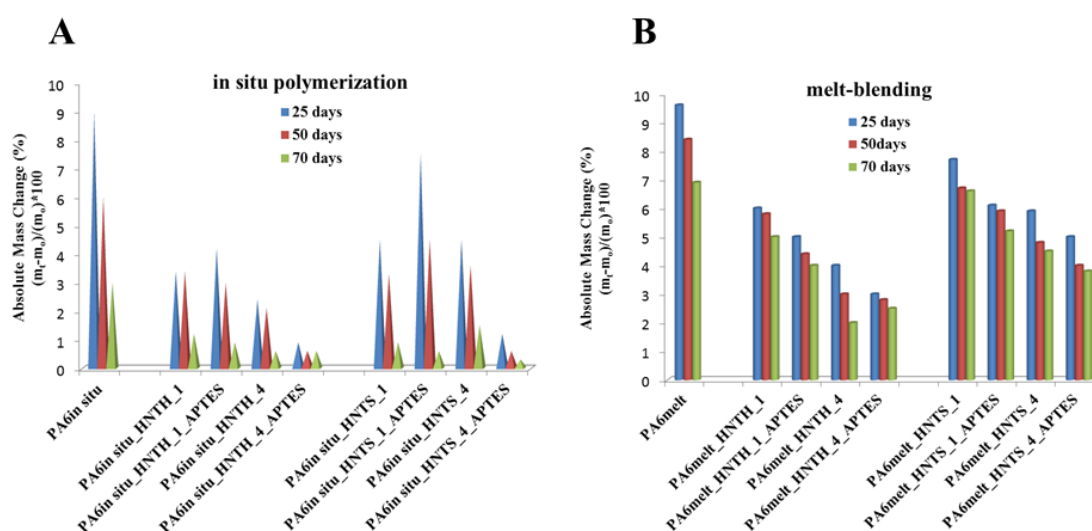


Figure 3.7 - Water absorption in A) PA6_{in situ}_HNT and B) PA6_{melt}_HNT nanocomposites.

Sample	weight (g)	weight (g)	weight (g)	weight (g)
	0 days	25 days	50 days	70 days
PA6 _{in situ}	14.39136	15.78833	14.53639	13.57776
PA6 _{in situ} _HNT _H _1	14.73355	14.75058	14.69237	13.93750
PA6 _{in situ} _HNT _s _1	14.26413	14.46217	14.40418	13.46581
PA6 _{in situ} _HNT _H _4	13.51417	13.80918	13.68247	13.21360
PA6 _{in situ} _HNT _s _4	13.62848	13.982774	13.65311	12.87875
PA6 _{in situ} _HNT _H _1_APTES	14.78595	15.00673	14.75758	14.03173
PA6 _{in situ} _HNT _s _1_APTES	12.53389	13.63417	12.62229	10.64601
PA6 _{in situ} _HNT _H _4_APTES	11.87400	11.90282	11.83065	11.82596
PA6 _{in situ} _HNT_4_APTES	12.81676	12.84718	12.78034	11.88061
PA6 _{melt}	12.51653	13.8525	12.98502	12.49943
PA6 _{melt} _HNT _H _1	12.27060	12.56998	12.35126	12.15381
PA6 _{melt} _HNT _s _1	12.83466	14.1918	12.82144	12.76107
PA6 _{melt} _HNT _H _4	12.00417	13.10212	12.95485	11.52131
PA6 _{melt} _HNT _s _4	12.20384	13.95077	13.00279	12.87224
PA6 _{melt} _HNT _H _1_APTES	11.78777	12.84055	12.49464	11.80357
PA6 _{melt} _HNT _s _1_APTES	12.11331	13.20204	13.06264	12.81039
PA6 _{melt} _HNT _H _4_APTES	13.47101	15.57738	15.4168	14.87602
PA6 _{melt} _HNT _s _4_APTES	12.42536	13.96671	13.2583	12.08642

Table 3.6 - Weight of the PA6_HNT nanocomposites collected during the hydrothermal ageing test at different times.

The effect of hydrothermal ageing on the rheological properties of PA6_HNT nanocomposites was studied. According to Figure 3.8 A,B, PA6_{in situ} nanocomposites with the 4% w/w of modified filler have higher complex viscosity values than all other samples thanks to the combined effect of the higher HNT loading and APTES presence that favours the dispersion of the inorganic filler inside the polymer matrix[76]. In the case of PA6_{melt} nanocomposites (Figure 3.9 A,B) their complex viscosity is not significantly affected by the addition of bare and modified HNT.

After ageing, the complex viscosity decreases in all the samples prepared (Figure 3.8 C,D and Figure 3.9 C,D) with the exception of the PA6_{in situ} samples prepared with the 4% of modified HNT (Figure 3.10). The complex viscosity decrease for PA6 and other polyamides after a long ageing has been widely reported in the literature[77] and can be ascribed to the plasticization effect of water: during hydrothermal ageing, water molecules are mainly absorbed by the amorphous phase where they interfere with the hydrogen bonds and break the secondary bonds between the PA6 groups. This induces an increase in the chain mobility and decreases consequently the complex viscosity. However, Figure 3.10 clearly shows that the decrease of PA6_{in situ} nanocomposites complex viscosities is only slightly present in the samples with modified HNT_H/HNT_s at

4% w/w, in good agreement with water uptake results. The observed difference in terms of complex viscosity between unaged and aged samples are not significant.

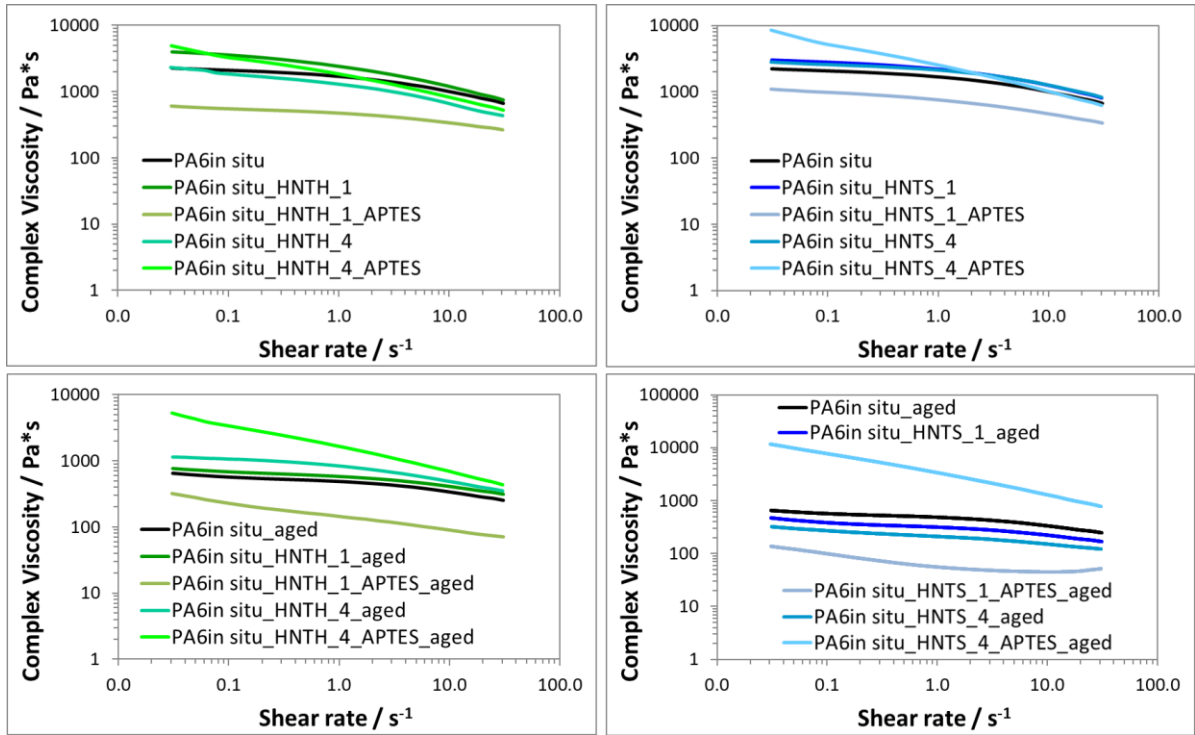


Figure 3.8 - Rheological curves of A) PA6_{in situ}_HNT_H, B) PA6_{in situ}_HNT_S, C) PA6_{in situ}_HNT_H_aged and D) PA6_{in situ}_HNT_S_aged samples.

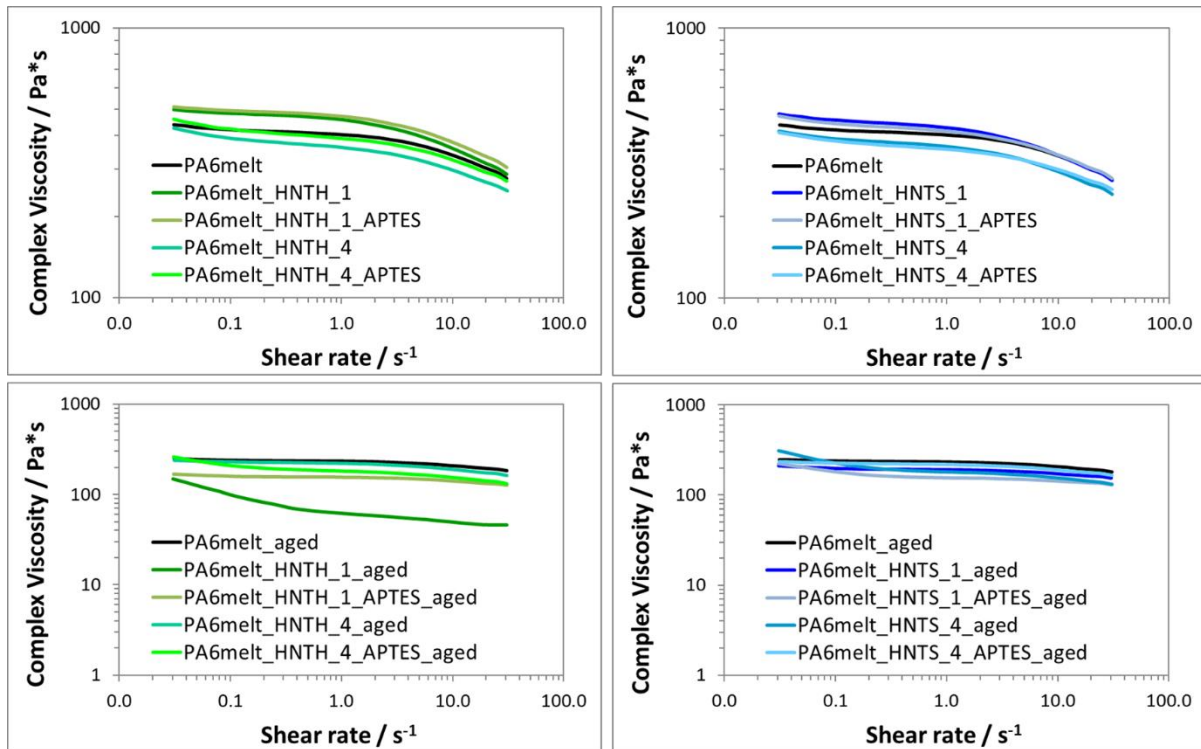


Figure 3.9 - Rheological curves of A) PA6_{melt}_HNT_H, B) PA6_{melt}_HNT_S, C) PA6_{melt}_HNT_H_aged and D) PA6_{melt}_HNT_S_aged samples.

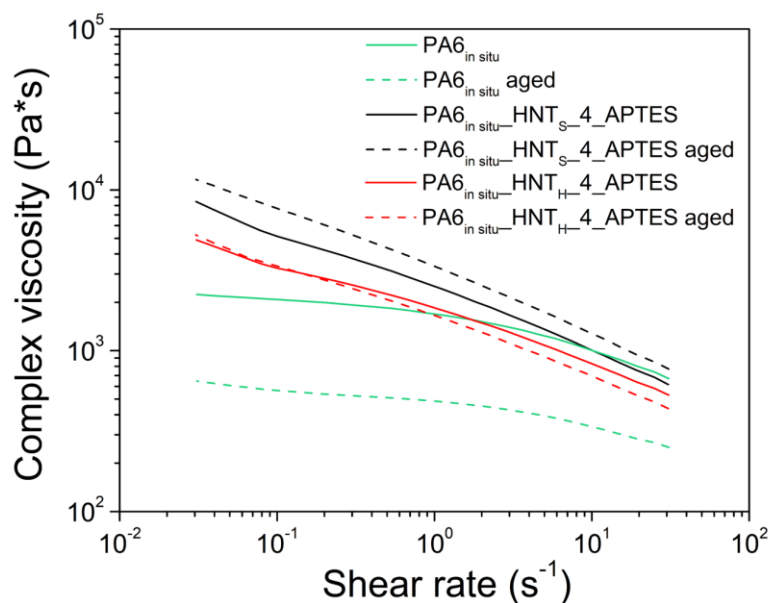


Figure 3.10 - Rheological behaviour of neat and APTES-functionalized HNT composites from in situ polymerization before and after hydrothermal ageing test.

Furthermore, the effect of the hydrothermal ageing on PA6-based nanocomposites can be highlighted by the evaluation of the number average molecular weight ($\overline{Mn}$) and molecular weight distribution (D) determined by SEC analyses (Table 3.7). It should be noted that samples were filtered with a 0.45 μm filter, therefore molecular weight data refer only to the part of the polymer present in solution. In particular, no significant decrease in molecular weight can be observed in samples from melt blending, pointing out to the absence of hydrolysis of polymer chains due to the filler surface hydroxylation. The lack of any significant effect might be explained, on one hand, with the drying step performed on the filler before incorporation, which removes physisorbed water that could lead to undesired hydrolysis reactions. On the other hand, surface hydroxylation of HNT is mainly related to the inner lumen, exposing $\text{Al}(\text{OH})_x$ groups, while the outer surface of the nanotubes mainly exposes Si-O-Si groups and silanol groups are present only as defects[78]. The lack of accessible surface hydroxyl groups limits the occurrence of hydrolysis phenomena of the polymer matrix. However, in good agreement with water uptake behaviour, a slight decrease in $\overline{Mn}$ data and broader D values are generally observed upon hydrothermal ageing as a result of degradation of PA6 chains[73]. This behaviour is shown by both neat PA6 and HNT-PA6 composite, with the notable exception of the nanocomposites prepared with higher loadings of modified fillers exhibiting almost unaltered parameters. For the latter samples, the sorption mechanism of water molecules is impeded by the presence of just 4% w/w of modified filler, which limits the diffusion of water molecules into the nanocomposites. As representative examples, Figure 3.11 compares the SEC curves, obtained before and after the hydrothermal test, of neat PA6 with those of PA6 nanocomposites prepared with the 4% w/w of modified filler.

Sample	SEC _{unaged}		SEC _{aged}	
	$\overline{Mn}$ (Da)	D	$\overline{Mn}$ (Da)	D
PA6 _{in situ}	57200	2.1	45800	2.4
PA6 _{in situ} _HNT _H _1	48600	2.1	32400	2.5
PA6 _{in situ} _HNT _S _1	56700	2.1	49300	2.5
PA6 _{in situ} _HNT _H _4	60400	2.0	42000	2.2
PA6 _{in situ} _HNT _S _4	62600	2.1	50300	2.6
PA6 _{in situ} _HNT _H _1_APTES	55600	2.1	45400	2.4
PA6 _{in situ} _HNT _S _1_APTES	54600	2.2	49900	2.6
PA6 _{in situ} _HNT _H _4_APTES	47800	2.3	41800	2.3
PA6 _{in situ} _HNT _S _4_APTES	60600	2.3	59000	2.3
PA6 _{melt}	43600	1.9	33100	2.2
PA6 _{melt} _HNT _H _1	40300	2.0	19300	2.5
PA6 _{melt} _HNT _S _1	40400	2.0	19700	2.5
PA6 _{melt} _HNT _H _4	42400	2.0	21000	2.3
PA6 _{melt} _HNT _S _4	42300	2.0	22100	2.3
PA6 _{melt} _HNT _H _1_APTES	40400	2.0	33100	2.2
PA6 _{melt} _HNT _S _1_APTES	39700	1.9	22300	2.2
PA6 _{melt} _HNT _H _4_APTES	42500	2.0	42000	2.0
PA6 _{melt} _HNT _S _4_APTES	39900	2.0	39800	2.0

Table 3.7 - Number average molecular weight ($\overline{Mn}$) and molecular weight distribution (D) data of PA6_HNT nanocomposites collected before and after the hydrothermal aging test.

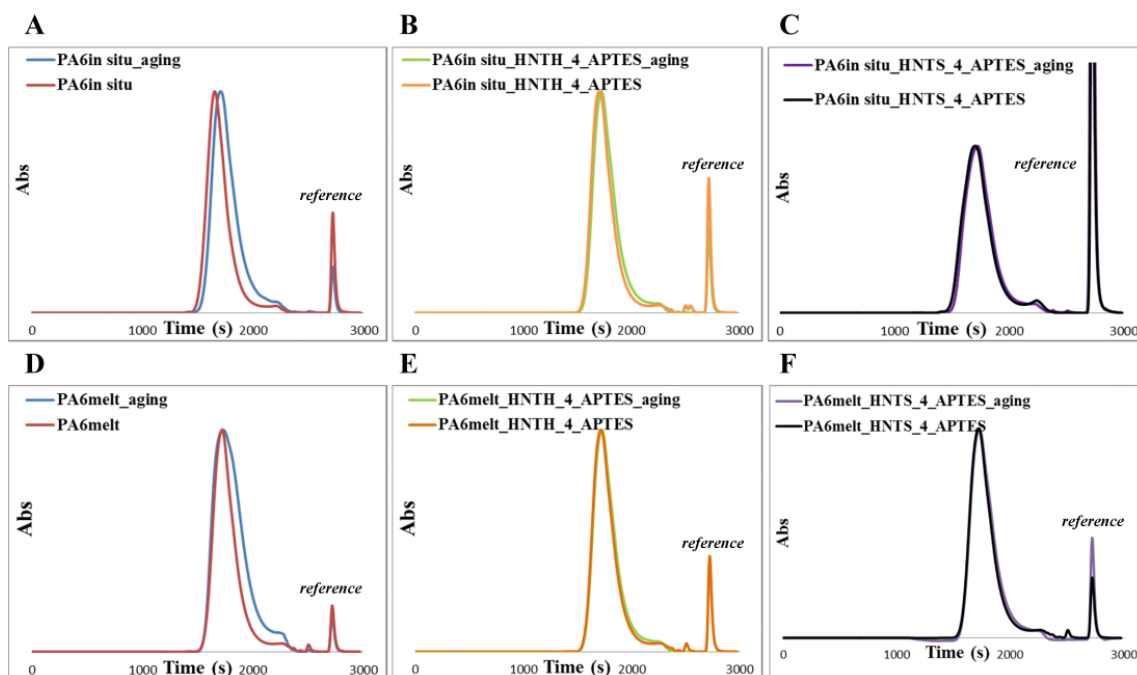


Figure 3.11 -SEC curves before and after the ageing test of A) PA6_{in situ}, B) PA6_{in situ}_HNT_H_4_APTES, C) PA6_{in situ}_HNT_S_4_APTES, D) PA6_{melt}, E) PA6_{melt}_HNT_H_4_APTES and F) PA6_{melt}_HNT_S_4_APTES nanocomposites.

Lastly, to evaluate the consequences of harsh ageing conditions on PA6 nanocomposites crystallization and melting temperatures, DSC measurements were conducted (Table 3.8). Comparing the thermal data obtained before and after the ageing test, it is worth noting that only the following samples showed minor changes in crystallization kinetics:

- PA6_{in situ}_HNT_H_4_APTES: χ_{c_unaged} : 14.0% *vs* χ_{c_aged} : 13.3%
- PA6_{in situ}_HNT_S_4_APTES: χ_{c_unaged} : 14.9% *vs* χ_{c_aged} : 14.9%
- PA6_{melt}_HNT_H_4_APTES: χ_{c_unaged} : 21.7% *vs* χ_{c_aged} : 21.1%
- PA6_{melt}_HNT_S_4_APTES: χ_{c_unaged} : 19.3% *vs* χ_{c_aged} : 20.3%.

Together with the reported presence of the γ crystalline form of PA6 even after the ageing test (Figure 3.12), this is a further evidence of the high hydrothermal stability of the nanocomposites prepared with 4% w/w of modified HNT.

Sample	cooling		2 nd heating		
	T _c (J/g)	ΔH_c (J/g)	T _m (J/g)	ΔH_f (J/g)	X _c (%)
PA6 _{in situ}	189.3	64.6	222.1	-61.7	25.7
PA6 _{in situ} _HNT _H _1	189.0	70.2	221.3	-61.6	25.7
PA6 _{in situ} _HNT _S _1	187.4	52.8	224.3	-47.7	19.9
PA6 _{in situ} _HNT _H _4	184.6	56.7	220.4	-47.3	19.7
PA6 _{in situ} _HNT _S _4	185.7	63.9	221.9	-59.5	24.8
PA6 _{in situ} _HNT _H _1_APTES	188.7	76.5	221.1	-48.0	28.0
PA6 _{in situ} _HNT _S _1_APTES	188.5	70.5	222.7	-42.9	17.9
PA6 _{in situ} _HNT _H _4_APTES	184.9	62.7	220.7	-31.9	13.3
PA6 _{in situ} _HNT _S _4_APTES	184.7	66.8	221.7	-35.7	14.9
PA6 _{melt}	190.5	62.9	222.8	-61.8	25.8
PA6 _{melt} _HNT _H _1	192.5	71.5	221.0	-51.1	21.3
PA6 _{melt} _HNT _S _1	191.6	69.9	221.5	-61.8	25.8
PA6 _{melt} _HNT _H _4	190.2	68.1	221.4	-40.1	16.7
PA6 _{melt} _HNT _S _4	190.7	65.0	222.0	-55.3	23.0
PA6 _{melt} _HNT _H _1_APTES	191.4	71.6	221.2	-59.8	24.9
PA6 _{melt} _HNT _S _1_APTES	192.7	80.4	220.8	-70.2	29.3
PA6 _{melt} _HNT _H _4_APTES	187.9	75.4	221.2	-50.6	21.1
PA6 _{melt} _HNT _S _4_APTES	188.8	67.0	222.8	-48.7	20.3

Table 3.8 - Differential scanning calorimetry data of PA6_HNT nanocomposites collected during cooling and second heating thermal steps after the hydrothermal ageing test.

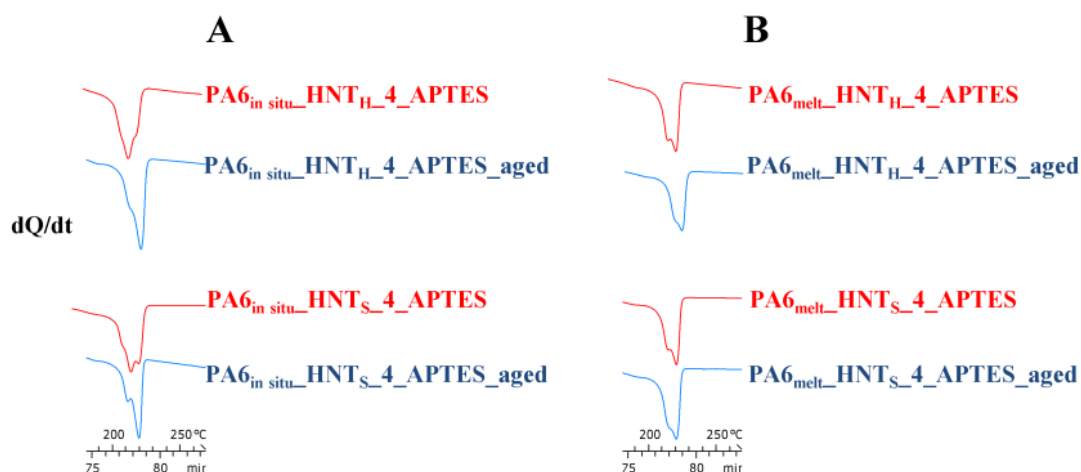


Figure 3.12 - Heating scans of A) PA6_{in situ}_HNT and B) PA6_{melt}_HNT nanocomposites prepared with the 4% w/w of modified HNT before and after the aging test.

3.3 Conclusions

In the field of shelf-life improvement for polymer materials, the development of PA6 nanocomposites characterized by enhanced moisture absorption resistance is always a pressing request due to the irreversible decay mechanisms subsequent to water molecules accumulation in the polymer matrix. In this context, the amino-silanization of HNT used as PA6 additives could be a way to create tailor-made materials with enhanced durability.

The purpose of this study was to investigate the influence of very low amounts, *i.e.* 1 and 4% w/w, of HNT on the hydrothermal ageing resistance of PA6 nanocomposites. The use of very low filler loadings provides multiple advantages, including lower alteration of the polymer appearance and a less extended polymer/filler interface where moisture accumulation may occur.

Crystal growth and type of nucleation in PA6 nanocomposites were studied on the basis of Avrami theory via isothermal crystallization studies, and of dynamic DSC evaluations, showing that the use of low HNT loading does not influence the homogenous PA6 crystal growth and, at the same time, adding HNT promotes the PA6 polymorphism, *i.e.* the formation of γ counterpart.

A multi-faceted approach was here employed by studying the physicochemical properties of differently sourced HNT, the filler functionalization, and last but not least, the dispersion technique, *i.e.* *in situ* polymerization and melt extrusion processes.

The HNT dispersion efficiency into the PA6 matrix was monitored by means of SEM/EDX analyses, showing clear differences between the two preparation methods: while HNT is homogeneously distributed via melt blending, *in situ* polymerization guarantees a better incorporation within the polymer matrix.

Both *in situ* and melt blending dispersion techniques showed that a lower surface area, a higher aspect ratio and a larger surface charge of HNT enhance filler incorporation and improve the final composite performances. It should be noted that the tested HNT

samples showed as well different structural properties in terms of crystallinity and phase impurity, which might affect the filler incorporation. These observations highlight the importance of a preliminary assessment of the physicochemical features of naturally sourced fillers, as relatively minor changes can notably affect the final composite properties.

The filler surface modification with aminosilanes plays a major role in the durability of the PA6_HNT nanocomposites, as determined by very long hydrothermal ageing exposure (1680 h). Notably, the nanocomposites with 4% w/w of modified HNT_H and HNT_S appear to be the most durable ones in terms of water uptake and rheological, molecular weights, molecular weight distribution and thermal properties change before and after the ageing test. This might be related to the ability of propyl chains present on HNT surface to hinder the diffusion of water molecules into the nanocomposites by increasing the hydrophobicity of the filler, enhancing the overall durability of the resulting nanocomposites.

The present research has led to a deeper comprehension of the macromolecular, thermal and rheological properties of HNT-based nanocomposites that can be successfully adopted as composite materials with improved resistance to hydrothermal ageing.

3.4 References

- [1] B. Wetzel, F. Hauptert, K. Friedrich, M.Q. Zhang, M.Z. Rong, Impact and wear resistance of polymer nanocomposites at low filler content, *Polym. Eng. Sci.* 42 (2002) 1919–1927. doi:10.1002/pen.11084.
- [2] S. Fu, Z. Sun, P. Huang, Y. Li, N. Hu, Some basic aspects of polymer nanocomposites: A critical review, *Nano Mater. Sci.* 1 (2019) 2–30. doi:10.1016/j.nanoms.2019.02.006.
- [3] A. Jacob, Carbon fibre and cars – 2013 in review, *Reinf. Plast.* 58 (2014) 18–19. doi:10.1016/S0034-3617(14)70036-0.
- [4] R. Stewart, Rebounding automotive industry welcome news for FRP, *Reinf. Plast.* 55 (2011) 38–44. doi:10.1016/S0034-3617(11)70036-4.
- [5] A.R. Horrocks, B.K. Kandola, P.J. Davies, S. Zhang, S.A. Padbury, Developments in flame retardant textiles - A review, *Polym. Degrad. Stab.* 88 (2005) 3–12. doi:10.1016/j.polymdegradstab.2003.10.024.
- [6] A.A. Mikhutkin, R.A. Kamyshinsky, T.K. Tenchurin, A.D. Shepelev, A.S. Orekhov, T.E. Grigoriev, V.G. Mamaguashvili, S.N. Chvalun, A.L. Vasiliev, Towards Tissue Engineering: 3D Study of Polyamide-6 Scaffolds, *Bionanoscience.* 8 (2018) 511–521. doi:10.1007/s12668-017-0493-0.
- [7] H.J. Song, Y. Chang, J.S. Lyu, M.Y. Yon, H. Lee, S.-J. Park, J.C. Choi, M. Kim, J. Han, Migration study of caprolactam from polyamide 6 sheets into food simulants, *Food Sci. Biotechnol.* 27 (2018) 1685–1689. doi:10.1007/s10068-018-0403-4.
- [8] W. Dong, P. Gijsman, Influence of temperature on the thermo-oxidative degradation of polyamide 6 films, *Polym. Degrad. Stab.* 95 (2010) 1054–1062.

doi:10.1016/j.polymdegradstab.2010.02.030.

- [9] P. Gijsman, G. Meijers, G. Vitarelli, Comparison of the UV-degradation chemistry of polypropylene, polyethylene, polyamide 6 and polybutylene terephthalate, *Polym. Degrad. Stab.* 65 (1999) 433–441. doi:10.1016/S0141-3910(99)00033-6.
- [10] D. Forsström, B. Terselius, Thermo oxidative stability of polyamide 6 films I. Mechanical and chemical characterisation, *Polym. Degrad. Stab.* 67 (2000) 69–78. doi:10.1016/S0141-3910(99)00122-6.
- [11] H. Shinzawa, J. Mizukado, Water absorption by polyamide (PA) 6 studied with two-trace two-dimensional (2T2D) near-infrared (NIR) correlation spectroscopy, *J. Mol. Struct.* 1217 (2020) 128389. doi:10.1016/j.molstruc.2020.128389.
- [12] I. Ksouri, O. De Almeida, N. Haddar, Long term ageing of polyamide 6 and polyamide 6 reinforced with 30% of glass fibers: physicochemical, mechanical and morphological characterization, *J. Polym. Res.* 24 (2017) 133. doi:10.1007/s10965-017-1292-6.
- [13] L. Sang, C. Wang, Y. Wang, W. Hou, Effects of hydrothermal aging on moisture absorption and property prediction of short carbon fiber reinforced polyamide 6 composites, *Compos. Part B Eng.* 153 (2018) 306–314. doi:10.1016/j.compositesb.2018.08.138.
- [14] D.P.N. Vlasveld, J. Groenewold, H.E.N. Bersee, S.J. Picken, Moisture absorption in polyamide-6 silicate nanocomposites and its influence on the mechanical properties, *Polymer (Guildf)*. 46 (2005) 12567–12576. doi:10.1016/j.polymer.2005.10.096.
- [15] V. Sabatini, H. Farina, L. Basilissi, G. Di Silvestro, M.A. Ortenzi, The Use of Epoxy Silanes on Montmorillonite: An Effective Way to Improve Thermal and Rheological Properties of PLA/MMT Nanocomposites Obtained via “in Situ” Polymerization, *J. Nanomater.* 2015 (2015). doi:10.1155/2015/418418.
- [16] M.K. Akkapeddi, Glass fiber reinforced polyamide-6 nanocomposites, *Polym. Compos.* 21 (2000) 576–585. doi:10.1002/pc.10213.
- [17] G. Wu, B. Li, J. Jiang, Carbon black self-networking induced co-continuity of immiscible polymer blends, *Polymer (Guildf)*. 51 (2010) 2077–2083. doi:10.1016/j.polymer.2010.03.007.
- [18] F.M. Uhl, Q. Yao, H. Nakajima, E. Manias, C.A. Wilkie, Expandable graphite/polyamide-6 nanocomposites, *Polym. Degrad. Stab.* 89 (2005) 70–84. doi:10.1016/j.polymdegradstab.2005.01.004.
- [19] F. Yang, Y. Ou, Z. Yu, Polyamide 6/silica nanocomposites prepared by in situ polymerization, *J. Appl. Polym. Sci.* 69 (1998) 355–361. doi:10.1002/(SICI)1097-4628(19980711)69:2<355::AID-APP17>3.0.CO;2-V.
- [20] Z. Xu, C. Gao, In situ polymerization approach to graphene-reinforced nylon-6 composites, *Macromolecules*. 43 (2010) 6716–6723. doi:10.1021/ma1009337.
- [21] K.P. Pramoda, T. Liu, Effect of moisture on the dynamic mechanical relaxation of polyamide-6/clay nanocomposites, *J. Polym. Sci. Part B Polym. Phys.* 42 (2004) 1823–1830. doi:10.1002/polb.20061.
- [22] A. Hassan, N.A. Rahman, R. Yahya, Moisture absorption effect on thermal, dynamic mechanical and mechanical properties of injection-molded short glass-fiber/polyamide 6,6 composites, *Fibers Polym.* 13 (2012) 899–906. doi:10.1007/s12221-012-0899-9.

- [23] M.K. Nazir M.S., Mohamad Kassim M.H., Mohapatra L., Gilani M.A., Raza M.R., Characteristic Properties of Nanoclays and Characterization of Nanoparticulates and Nanocomposites, in: Springer (Ed.), *Nanoclay Reinf. Polym. Compos.*, 2016: pp. 35–55.
- [24] L. Basilissi, G. Di Silvestro, H. Farina, M.A. Ortenzi, Synthesis and characterization of PLA nanocomposites containing nanosilica modified with different organosilanes I. Effect of the organosilanes on the properties of nanocomposites: Macromolecular, morphological, and rheologic characterization, *J. Appl. Polym. Sci.* 128 (2013) 1575–1582. doi:10.1002/app.38324.
- [25] P. V. Kodgire, A.R. Bhattacharyya, S. Bose, N. Gupta, A.R. Kulkarni, A. Misra, Control of multiwall carbon nanotubes dispersion in polyamide6 matrix: An assessment through electrical conductivity, *Chem. Phys. Lett.* 432 (2006) 480–485. doi:10.1016/j.cplett.2006.10.088.
- [26] W. De Zhang, L. Shen, I.Y. Phang, T. Liu, Carbon nanotubes reinforced nylon-6 composite prepared by simple melt-compounding, *Macromolecules.* 37 (2004) 256–259. doi:10.1021/ma035594f.
- [27] M.R. Ayatollahi, S. Shadlou, M.M. Shokrieh, M. Chitsazzadeh, Effect of multi-walled carbon nanotube aspect ratio on mechanical and electrical properties of epoxy-based nanocomposites, *Polym. Test.* 30 (2011) 548–556. doi:10.1016/j.polymertesting.2011.04.008.
- [28] B. Pan, N. Li, G. Chu, F. Wei, J. Liu, J. Zhang, Y. Zhang, Tribological investigation of MC PA6 reinforced by boron nitride of single layer, *Tribol. Lett.* 54 (2014) 161–170. doi:10.1007/s11249-014-0324-1.
- [29] W.L. Song, P. Wang, L. Cao, A. Anderson, M.J. Meziani, A.J. Farr, Y.P. Sun, Polymer/boron nitride nanocomposite materials for superior thermal transport performance, *Angew. Chemie - Int. Ed.* 51 (2012) 6498–6501. doi:10.1002/anie.201201689.
- [30] M. De Monte, E. Moosbrugger, M. Quaresimin, Influence of temperature and thickness on the off-axis behaviour of short glass fibre reinforced polyamide 6.6 - Cyclic loading, *Compos. Part A Appl. Sci. Manuf.* 41 (2010) 1368–1379. doi:10.1016/j.compositesa.2010.02.004.
- [31] G. Ozkoc, G. Bayram, E. Bayramli, Effects of polyamide 6 incorporation to the short glass fiber reinforced ABS composites: An interfacial approach, *Polymer (Guildf).* 45 (2004) 8957–8966. doi:10.1016/j.polymer.2004.10.026.
- [32] E. Reynaud, T. Jouen, C. Gauthier, G. Vigier, J. Varlet, Nanofillers in polymeric matrix: A study on silica reinforced PA6, *Polymer (Guildf).* 42 (2001) 8759–8768. doi:10.1016/S0032-3861(01)00446-3.
- [33] R. Gensler, P. Gröppel, V. Muhrer, N. Müller, Application of nanoparticles in polymers for electronics and electrical engineering, *Part. Part. Syst. Charact.* 19 (2002) 293–299. doi:10.1002/1521-4117(200211)19:5<293::AID-PPSC293>3.0.CO;2-N.
- [34] M. Liu, Z. Jia, D. Jia, C. Zhou, Recent advance in research on halloysite nanotubes-polymer nanocomposite, *Prog. Polym. Sci.* 39 (2014) 1498–1525. doi:10.1016/j.progpolymsci.2014.04.004.
- [35] Y. Lvov, W. Wang, L. Zhang, R. Fakhrullin, Halloysite Clay Nanotubes for Loading

and Sustained Release of Functional Compounds, *Adv. Mater.* 28 (2016) 1227–1250. doi:10.1002/adma.201502341.

- [36] G. Tari, I. Bobos, C.S.F. Gomes, J.M.F. Ferreira, Modification of surface charge properties during kaolinite to halloysite-7Å transformation, *J. Colloid Interface Sci.* 210 (1999) 360–366. doi:10.1006/jcis.1998.5917.
- [37] D. Rawtani, Y.K. Agrawal, Multifarious applications of halloysite nanotubes: A review, *Rev. Adv. Mater. Sci.* 30 (2012) 282–295.
- [38] D.G. Shchukin, G.B. Sukhorukov, R.R. Price, Y.M. Lvov, Halloysite nanotubes as biomimetic nanoreactors, *Small.* 1 (2005) 510–513. doi:10.1002/sml.200400120.
- [39] B. Guo, Q. Zou, Y. Lei, D. Jia, Structure and performance of polyamide 6/halloysite nanotubes nanocomposites, *Polym. J.* 41 (2009) 835–842. doi:10.1295/polymj.PJ2009110.
- [40] D.C.O. Marney, L.J. Russell, D.Y. Wu, T. Nguyen, D. Cramm, N. Rigopoulos, N. Wright, M. Greaves, The suitability of halloysite nanotubes as a fire retardant for nylon 6, *Polym. Degrad. Stab.* 93 (2008) 1971–1978. doi:10.1016/j.polymdegradstab.2008.06.018.
- [41] U.A. Handge, K. Hedicke-Höchstötter, V. Altstädt, Composites of polyamide 6 and silicate nanotubes of the mineral halloysite: Influence of molecular weight on thermal, mechanical and rheological properties, *Polymer (Guildf).* 51 (2010) 2690–2699. doi:10.1016/j.polymer.2010.04.041.
- [42] K. Prashantha, H. Schmitt, M.F. Lacrampe, P. Krawczak, Mechanical behaviour and essential work of fracture of halloysite nanotubes filled polyamide 6 nanocomposites, *Compos. Sci. Technol.* 71 (2011) 1859–1866. doi:10.1016/j.compscitech.2011.08.019.
- [43] B. Lecouvet, J.G. Gutierrez, M. Sclavons, C. Bailly, Structure-property relationships in polyamide 12/halloysite nanotube nanocomposites, *Polym. Degrad. Stab.* 96 (2011) 226–235. doi:10.1016/j.polymdegradstab.2010.11.006.
- [44] K. Prashantha, M.F. Lacrampe, P. Krawczak, Highly dispersed polyamide-11/halloysite nanocomposites: Thermal, rheological, optical, dielectric, and mechanical properties, *J. Appl. Polym. Sci.* 130 (2013) 313–321. doi:10.1002/app.39160.
- [45] J. Tung, R.K. Gupta, G.P. Simon, G.H. Edward, S.N. Bhattacharya, Rheological and mechanical comparative study of in situ polymerized and melt-blended nylon 6 nanocomposites, *Polymer (Guildf).* 46 (2005) 10405–10418. doi:10.1016/j.polymer.2005.08.043.
- [46] A. Bergeret, L. Ferry, P. Ienny, Influence of the fibre/matrix interface on ageing mechanisms of glass fibre reinforced thermoplastic composites (PA-6,6, PET, PBT) in a hygrothermal environment, *Polym. Degrad. Stab.* 94 (2009) 1315–1324. doi:10.1016/j.polymdegradstab.2009.04.009.
- [47] A. Bergeret, I. Pires, M.P. Foulc, B. Abadie, L. Ferry, A. Crespy, The hygrothermal behaviour of glass-fibre-reinforced thermoplastic composites: A prediction of the composite lifetime, *Polym. Test.* 20 (2001) 753–763. doi:10.1016/S0142-9418(01)00030-7.
- [48] R. Seltzer, P.M. Frontini, Y.W. Mai, Effect of hygrothermal ageing on morphology and indentation modulus of injection moulded nylon 6/organoclay nanocomposites,

Compos. Sci. Technol. 69 (2009) 1093–1100. doi:10.1016/j.compscitech.2009.01.029.

- [49] E.J.H.S.R.C. Schulz, A new method for gel permeation chromatography of polyamides, *Macromol. Rapid Commun.* 1 (1980) 397–402.
- [50] H. Piao, Influence of water absorption on the mechanical properties of discontinuous carbon fiber reinforced polyamide 6, (2019).
- [51] J.M. Falcón, T. Sawczen, I.V. Aoki, Dodecylamine-Loaded Halloysite Nanocontainers for Active Anticorrosion Coatings, *Front. Mater.* 2 (2015) 1–13. doi:10.3389/fmats.2015.00069.
- [52] E. Joussein, S. Petit, J. Churchman, B. Theng, D. Righi, B. Delvaux, Halloysite clay minerals – a review, *Clay Miner.* 40 (2005) 383–426. doi:10.1180/0009855054040180.
- [53] T. Taroni, D. Meroni, K. Fidecka, D. Maggioni, M. Longhi, S. Ardizzone, Halloysite nanotubes functionalization with phosphonic acids: Role of surface charge on molecule localization and reversibility, *Appl. Surf. Sci.* 486 (2019) 466–473. doi:10.1016/j.apsusc.2019.04.264.
- [54] D. Meroni, L. Lo Presti, G. Di Liberto, M. Ceotto, R.G. Acres, K.C. Prince, R. Bellani, G. Soliveri, S. Ardizzone, A Close Look at the Structure of the TiO₂-APTES Interface in Hybrid Nanomaterials and Its Degradation Pathway: An Experimental and Theoretical Study, *J. Phys. Chem. C.* 121 (2017) 430–440. doi:10.1021/acs.jpcc.6b10720.
- [55] K. Prashantha, M.F. Lacrampe, P. Krawczak, Processing and characterization of halloysite nanotubes filled polypropylene nanocomposites based on a masterbatch route: effect of halloysites treatment on structural and mechanical properties, *Express Polym. Lett.* 5 (2011) 295–307. doi:10.3144/expresspolymlett.2011.30.
- [56] T.D. Fornes, D.R. Paul, Crystallization behavior of nylon 6 nanocomposites, *Polymer (Guildf).* 44 (2003) 3945–3961. doi:10.1016/S0032-3861(03)00344-6.
- [57] L.T. S.Gogolewski, Study on crystallization of nylon-6 (polycapramide): Part 2. Effect of molecular weight on isothermal crystallization kinetics, *Polymer (Guildf).* 12 (1971) 629–641. doi:10.1016/0032-3861(71)90071-1.
- [58] G.G.D.G.R. Zannetti, Influence of nucleating agents on the crystallization of 6-polyamide, *Die Makromol. Chemie.* 179 (1978) 231–245. doi:10.1002/macp.1978.021790123.
- [59] Y. P. Khanna A. C. Reimschuessel A. Banerjee C. Altman, Memory effects in polymers. II. Processing history vs. crystallization rate of nylon 6—observation of phenomenon and product behavior, *Polym. Eng. Sci.* 28 (1988) 1600–1606. doi:10.1002/pen.760282405.
- [60] B.O. Reid, M. Vadlamudi, A. Mamun, H. Janani, H. Gao, W. Hu, R.G. Alamo, Strong memory effect of crystallization above the equilibrium melting point of random copolymers, *Macromolecules.* 46 (2013) 6485–6497. doi:10.1021/ma400839d.
- [61] A. Wurm, M. Ismail, B. Kretschmar, D. Pospiech, C. Schick, Retarded crystallization in polyamide/layered silicates nanocomposites caused by an immobilized interphase, *Macromolecules.* 43 (2010) 1480–1487. doi:10.1021/ma902175r.
- [62] B. Guo, Q. Zou, Y. Lei, M. Du, M. Liu, D. Jia, Crystallization behavior of polyamide 6/halloysite nanotubes nanocomposites, *Thermochim. Acta.* 484 (2009) 48–56.

doi:10.1016/j.tca.2008.12.003.

- [63] C. Caamaño, B. Grady, D.E. Resasco, Influence of nanotube characteristics on electrical and thermal properties of MWCNT/polyamide 6,6 composites prepared by melt mixing, *Carbon* N. Y. 50 (2012) 3694–3707. doi:10.1016/j.carbon.2012.03.043.
- [64] A. Kiziltas, D.J. Gardner, Y. Han, H.S. Yang, Dynamic mechanical behavior and thermal properties of microcrystalline cellulose (MCC)-filled nylon 6 composites, *Thermochim. Acta*. 519 (2011) 38–43. doi:10.1016/j.tca.2011.02.026.
- [65] E.M.Bradbury; L.Brown; A.Elliott; D.A.D.Parry, The structure of the gamma form of polycaproamide (Nylon 6), *Polymer (Guildf)*. 6 (1965) 465–482. doi:10.1016/0032-3861(65)90032-7.
- [66] D.M. Lincoln, R.A. Vaia, Z.G. Wang, B.S. Hsiao, R. Krishnamoorti, Temperature dependence of polymer crystalline morphology in nylon 6/montmorillonite nanocomposites, *Polymer (Guildf)*. 42 (2001) 09975–09985. doi:10.1016/s0032-3861(01)00542-0.
- [67] M. Porubská, O. Szöllös, A. Kóňová, I. Janigová, M. Jašková, K. Jomová, I. Chodák, FTIR spectroscopy study of polyamide-6 irradiated by electron and proton beams, *Polym. Degrad. Stab.* 97 (2012) 523–531. doi:10.1016/j.polymdegradstab.2012.01.017.
- [68] M. Zammarano, S. Bellayer, J.W. Gilman, M. Franceschi, F.L. Beyer, R.H. Harris, S. Meriani, Delamination of organo-modified layered double hydroxides in polyamide 6 by melt processing, *Polymer (Guildf)*. 47 (2006) 652–662. doi:10.1016/j.polymer.2005.11.080.
- [69] LinSangChuoWangYingyingWangWenbinHou, Effects of hydrothermal aging on moisture absorption and property prediction of short carbon fiber reinforced polyamide 6 composites, *Compos. Part B Eng.* 153 (2018) 306–314. doi:10.1016/j.compositesb.2018.08.138.
- [70] Y. Lei, J. Zhang, T. Zhang, H. Li, Water diffusion in carbon fiber reinforced polyamide 6 composites: Experimental, theoretical, and numerical approaches, *J. Reinf. Plast. Compos.* 38 (2019) 578–587. doi:10.1177/0731684419835034.
- [71] G.L. Lihua Gao, Lin Ye, Long-Term Hydrothermal Aging Behavior and Life-Time Prediction of Polyamide 6, *J. Macromol. Sci. Part B.* 54 (2015) 239–252. doi:10.1080/00222348.2014.1001660.
- [72] G.L. Ruiguang Li, Lin Ye, Long-Term Hydrothermal Aging Behavior and Aging Mechanism of Glass Fibre Reinforced Polyamide 6 Composites, *J. Macromol. Sci. Part B.* 57 (2017) 67–82. doi:10.1080/00222348.2018.1432174.
- [73] N. Chaupart, G. Serpe, J. Verdu, Molecular weight distribution and mass changes during polyamide hydrolysis, *Polymer (Guildf)*. 39 (1998) 1375–1380. doi:10.1016/S0032-3861(97)00414-X.
- [74] N. Kim, D.H. Shin, Y.T. Lee, Effect of silane coupling agents on the performance of RO membranes, *J. Memb. Sci.* 300 (2007) 224–231. doi:10.1016/j.memsci.2007.05.039.
- [75] J.R. Shallenberger, E.E. Metwalli, C.G. Pantano, F.N. Tuller, D.F. Fry, Adsorption of polyamides and polyamide-silane mixtures at glass surfaces, *Surf. Interface Anal.* 35 (2003) 667–672. doi:10.1002/sia.1589.

- [76] J.C. S.G. Pardo, C. Bernal, A. Ares, M.J. Abad, Rheological, Thermal, and Mechanical Characterization of Fly Ash-Thermoplastic Composites With Different Coupling Agents, *Polym. Compos.* 31 (2012) 1722–1730. doi:10.1002/pc.20962.
- [77] S. BERLAND, B. LAUNAY, Rheological properties of wheat flour doughs in steady and dynamic shear: effect of water content and some additives, *Cereal Chem.* 72 (1995) 48–52.
- [78] P. Yuan, D. Tan, F. Annabi-Bergaya, Properties and applications of halloysite nanotubes: recent research advances and future prospects, *Appl. Clay Sci.* 112–113 (2015) 75–93. doi:10.1016/j.clay.2015.05.001.

PART II

TiO₂ porous films

INTRODUCTION

Titanium dioxide is a widely diffused metal oxide. Largely adopted, since the beginning of its commercialization in the first years of the twentieth century, in many products such as paints, plastics, personal care and food products due to its white colour and high refractive index. Its scientific appeal would be uncovered only in 1972: that year, Fujishima and Honda published a ground-breaking paper in which they described for the first time the photocatalytic splitting of water into oxygen and hydrogen molecules by UV light irradiation on a TiO₂ electrode[1]. From then on, a gargantuan amount of research on the photocatalytic activity of TiO₂ in a wide number of reactions has been performed and even today continues to be one of the most actively discussed topics in the scientific community.

This is confirmed by the fact that up to now, among all the available photocatalysts, TiO₂ remains the most adopted one. The reasons behind this diffusion are its abundance, stability, high activity and bands positioning[2,3]. Moreover, it can be readily synthesised on a lab scale through a large number of different low-cost routes, such as sol-gel, hydrothermal, solvothermal and pyrolysis procedures. This allows to finely tune its morphology, obtaining both nano and micro structured materials, be them powders or films, designed to answer to the most disparate issues, from pollution remediation, to organic heterogeneous catalysis to functional coatings.

II.1 General properties

II.1.1 Structural properties

Titanium is the world's fourth most abundant metal, exceeded only by aluminium, iron, and magnesium, and the ninth most abundant element, constituting about 0.63% of the earth's crust[17].

Titanium dioxide can assume three main different crystalline forms: rutile, anatase and brookite[4]. The differences between these polymorphs extend beyond the mere crystallographic habit and involve other physicochemical properties, including band-gap width, surface structure, density and refractive index. Moreover, their photocatalytic activity shows great disparity, as well as the relative ease at which they can be

synthesised on a lab-scale[4]. Other crystallographic structures can be achieved through specific high-pressure and high-temperature synthetic procedures[5].

Rutile, from the Latin “rutilus” which means red, is the most diffused natural TiO_2 polymorph, found as a side mineral in metamorphic and igneous rocks formed at high pressure and temperature. Having the lowest cell volume amongst the three polymorphs, rutile is the favoured TiO_2 crystalline manifestation in such environments[6]. It owes its name to the reddish-brown colour it assumes in certain mineral species, which is caused by the presence of up to 10% Fe and significant amounts of other impurities. The hardness of Rutile sits between 6 and 6.5 on the Mohs scale and its density is about 4.23 g cm^{-3} [7]. Since its refractive index is particularly high, around 2.95, it is largely adopted as a pigment in paints. It has a tetragonal cell structure (space group: $P4_2/mnm$), where Ti(IV) d^0 ions are placed at the centre of TiO_6 distorted octahedral units (every Ti ion is surrounded by 6 O ions and every O by three Ti at the vertexes of a nearly equilateral triangle). Every octahedron shares two opposite edges with neighbours, thus forming rows of octahedrons parallel to the [001] direction. Such an arrangement leads to the formation of channels along the [001] direction.

Anatase derives its name from the Greek word *ανάτασις*, which means to stretch, extend, probably due to the elongated appearance of its bipyramidal crystals[6]. Its structure also earned it the name octahedrite in the past, as its units are easily mistakable for elongated octahedrons. In nature, anatase usually forms minuscule crystals exhibiting a wide range of hues, from blue to yellow, depending on the type of impurities present in the mineral formation. Its hardness is slightly lower than rutile, orbiting around 6 on the Mohs scale, as is its density, which is 3.8 g cm^{-3} [8]. This is linked to the fact that it is thermodynamically metastable, but highly kinetically favoured at lower temperatures ($< 500^\circ\text{C}$) and pressures, which is coherent with its less compact structure[9]. Anatase cells are tetragonal (space group: $I4_1/amd$), as rutile, but, contrary to its denser counterpart, they contain 4 formula units instead of 2. Ti(IV) ions are still inscribed in TiO_6 octahedrons, but their shape is even more distorted than for rutile, as each one shares 4 edges and 4 corners with neighbouring units. This conformation leads to an increase in Ti-Ti distances and to a reduction of O-O ones. Channels are present also in the crystalline habit of anatase and are even more abundant, allowing the inclusion of either cations or anions during the synthetic stage. Furthermore, it is reported as the most photocatalytically active polymorph of TiO_2 [2].

Finally, brookite, named after the English mineralogist Henry James Brooke, is the rarest and least stable of all three polymorphs. It is also the less investigated one. It crystallizes in small tubular or platy crystals, characterised from colours going from pink to brown and the lowest hardness amongst the three, around 5.5 – 6 on the Mohs scale[9]. Its density sits between that of rutile and anatase, at 4.0 g cm^{-3} [10]. In addition to the features setting it apart from the other two polymorphs, its crystal structure is the most complex one: it crystallizes with an orthorhombic crystallographic system (space group: $Pbca$) and the unit cells contain 8 formula units. TiO_6 octahedra show a marked distortion, as all atomic distances are different and each octahedral unit shares three edges with the neighbouring ones. As in rutile, there are channels along the [001] direction, capable of housing small ions, like H and Li.

II.1.2 Thermodynamic properties

As mentioned before, rutile is the most thermodynamically stable TiO_2 polymorph at all temperatures and at pressures up to 60 kbar, after which point TiO_2 -II, also known as columbite, becomes the thermodynamically favourite phase, due to its dense $\alpha\text{-PbO}_2$ -like crystal structure[11]. Bulk anatase and brookite are therefore metastable polymorphs, but the small differences in Gibbs free energy ($4\text{--}20\text{ kJ mol}^{-1}$) between the three phases suggest that, at normal pressure and low temperatures, all three phases can be considered to be relatively stable. As their Gibbs free energy differs so slightly from one another, the relative phase stability of TiO_2 may be reversed by changing particle size. As particles become smaller, their surface/volume ratio increases, raising the relevance of surface-related energetic components, namely surface free energy and surface stress[12]. Surface free energy is defined as the reversible work per unit area involved in forming a new surface of a substance, for instance by cleavage, while the reversible work per unit area required to elastically stretch a surface is called surface stress[13]. Following the square-cube law, as particle size decreases, these two factors become more and more impactful in the definition of the global free energy, up to the point of even reverting phase stability when the diameter of particles reaches a sufficiently low value. For example, while macroscopic anatase has lower thermodynamic stability compared to rutile, it has also lower surface energy: therefore, below a certain size threshold, its global free energy will result lower than rutile because surface components are less impactful for anatase than they are for rutile[14]. Due to this phenomenon, each of the three polymorphs will be the most thermodynamically stable phase within specific size intervals: anatase is the favoured phase up to 14 nm, between 14 and 35 nm brookite is, while rutile is the most stable from 35 nm upwards[14]. While this is the general rule, different occurrences have been experimentally reported.

As stated before, while it is true that rutile is the most thermodynamically favoured polymorph at ambient conditions, the enthalpy of the phase transformation from anatase to rutile is very low. Anatase is kinetically favoured, so that at room temperature the phase shift is so slow it practically does not occur. For bulk TiO_2 , it reaches a measurable rate only at temperatures over 600°C [15]. This transformation, achieved by increased temperature or pressure, is influenced by several factors, like the concentration of lattice and surface defects and particle size.

II.1.3 Photocatalytic properties

Photocatalysis is a physicochemical process in which a semiconductor material, taking the role of *photocatalyst*, promotes oxidation or reduction reactions upon irradiation with light of suitable energy[1]. In a solid state material electrons can occupy two different groups of energy levels, also called bands: the lower energy band is called the *valence band* and the large majority of the electrons are confined within its energy boundaries when they are not subjected to any particular stimulus. The higher energy band is called the *conduction band* and represents the energy state of electrons promoted through external energy inputs. These two electronic bands are divided by an energy gap, called *band gap*, which comprises energy levels forbidden to the electrons, making

them unavailable. It is the extent of said band gap that determines whether materials are considered conductors, semiconductors or insulators. Conductors are characterized by the absence of a band gap, granting the continuity of permitted energy levels. On the other hand, the distinction between semiconductors and insulators is more arbitrary, as it depends on the promotability of valence electrons: indeed, a semiconductor could be defined as a material which band gap is not so wide as to make it impossible to promote electrons to the conduction band upon light absorption. Generally, this limit is considered to sit around a band gap of 4 eV.

In order to activate a semiconductor, light radiation of the appropriate level, *i.e.* equal or greater than the band gap, is needed[16]. Upon light irradiation with photons of adequate wavelength, their energy causes the semiconductor electrons to be promoted into the conduction band, leaving an *electron hole*, or h^+ , in the valence band. This hole can be considered as a positively-charged charge carrier. This simultaneously generates two charge carriers bearing opposite charge, generally referred to as *electron-hole couple*, or e^-/h^+ . This phenomenon can occur in metals as well, but unlike conductors, for which the continuum of inter-band energetic levels causes an almost instantaneous electron/hole recombination, the band gap of semiconductors grants the e^-/h^+ pair a sufficient lifetime, in the order of nanoseconds, allowing the transfer of the photoexcited electron or hole to a reagent adsorbed at the catalyst surface. There they can promote a large number of reactions, either reducing or oxidizing molecules, which thus act as electron acceptors and electron donors respectively. Redox reactions can develop directly between the photocatalyst surface and the molecule or by means of highly reactive, mostly radical, species. Typically, during the photocatalytic process by TiO_2 , oxidation reactions are usually led by hydroxyl radicals, $\cdot OH$, and superoxide radical anions, $\cdot O_2^-$, together with electron holes. Depending on the adopted semiconductor, the direct action of electrons and holes on H_2O and/or O_2 molecules, generally the two main sources of oxidizing and radical species, can form several other oxidative agents that may attack the target molecule. Unfortunately, photocatalysis suffers from low quantum yields, expressed as the ratio between electron transfer phenomena and number of irradiating photons. This is caused by the high probability of e^-/h^+ recombination phenomena, occurring either at the semiconductor surface after migrating, or at bulk sites[17].

Other than the extension of the band gap, the other paramount parameter guiding the selection of an adequate semiconductor material for photocatalysis is the relative position of the electronic bands, which determines which kind of reaction can be carried out[18]. In this regard, the higher is the energy level of the conduction band minimum, *i.e.* the lower its potential compared to that of water reduction (H_2/H_2O), the stronger the reductive potential of photopromoted electrons will result. *Vice versa*, the lower the energy level of the valence band maximum, *i.e.* the higher its potential compared to that of water oxidation (O_2/H_2O or even $\cdot OH/H_2O$), the stronger the oxidative potential of electron holes formed in the valence band. Therefore, semiconductors can promote either only reduction, only oxidation or both kind of redox reactions, along with the formation of several radical species, depending on the energy levels of their valence and conduction bands. For these reasons, different semiconductors can prove more or less suitable for different photocatalytic reactions, like water splitting, oxidation of organic species and methane steam reforming to name a few.

TiO₂ has a band gap ranging from 3.0 eV to 3.3 eV, depending on the considered polymorph[18]. From a mere thermodynamic point of view, the conduction band minimum of TiO₂, which is situated above the potential of the H₂/H₂O redox couple (*ca.* −0.3 V *vs.* NHE), could promote water reduction, although this capability might be reduced by the extent of overpotentials[19], always to be considered. Conversely, its valence band maximum, sitting below the potential of both the O₂/H₂O and [•]OH/H₂O redox couples (*ca.* +2.9 V *vs.* NHE), allows it to promote oxidation reactions, such as the oxidative degradation of organics. For this reason, photogenerated holes often combine with hydroxyl species adsorbed at the TiO₂ surface, giving rise to hydroxyl radicals, which are active intermediates in the oxidation of other substances. The interfacial charge transfer is more effective when reagents are pre-adsorbed on the catalyst surface.

Other than the intrinsic electronic structure of the material, the photocatalytic activity of TiO₂ is strongly affected by multiple physicochemical factors, pertaining to both the structural and the morphological features of the photocatalyst. Phase composition, degree of crystallinity, surface area, pore volume and size distribution, surface hydration, surface charge, defectivity and presence of impurities are only some of the parameters responsible for the photocatalytic activity of TiO₂[20,21]. Most, if not all of them, can be tuned through the synthetic procedure adopted for the preparation of the sample. It goes without saying that one of the most crucial factors to (photo)catalytic activity is represented by surface area. Higher surface areas almost always translate to higher photocatalytic activity, for the simple fact that the area available for reagent adsorption is larger. It is for this reason that most research works focus on nanometric TiO₂ powders, as their specific surface area can easily reach over several hundred square metres per gram.

Aside from its effect on specific surface area, particle size also affects the band gap of semiconductors. Interestingly, it seems that two opposite phenomena are at play when reducing particle size, which can lead to both an increase and a decrease of the band gap extent. On one hand, smaller particles possess an increasing number of surface defects, which generate deep or shallow traps near the band edge, *de facto* reducing the band gap and causing a red shift in the absorption spectrum[22]. On the other hand, when the extension of a semiconductor material leaves the macroscopic level of the bulk and approaches that of Bohr radius, the space available for charge carriers to migrate can no longer be approximated to infinity and size quantization (Q-size) effects arise[23]: in a quantum sized semiconductor particle, electrons and holes are spatially confined in a potential well, unable to experience the electronic delocalization that occurs in the bulk phase. In this way, Q-size effects tend to increase the band gap of ultra-fine semiconductor powders, opposing the effect of surface defects. Several authors reported both red and blue shifts of the band gap as a function of particle dimensions[24,25]. Lin et al. reported that the band gap of anatase decreased monotonically from 3.239 to 3.173 eV when the particle size went from 29 to 17 nm and then rose again from 3.173 to 3.289 eV as the particle size further diminished from 17 to 3.8 nm[26].

While, as stated above, defects can lower the band gap causing absorption red shift, a high degree of crystallinity of the semiconductor material is equally important: bulk defects act as traps for charge carriers, causing the formation of localized electronic states. As crystallinity rises, the mobility of charge carriers increases, hence promoting

the photocatalytic activity[27]. This is why, generally, amorphous samples show reduced activity compared to crystalline ones. Nonetheless, surface defects provide a beneficial effect, as they trap electrons and holes at the photocatalyst surface, effectively prolonging their proximity to adsorbed reagent species, thus increasing charge transfer efficiency[22,28]. To maximise the benefits of both phenomena, the surface of highly crystalline TiO_2 particles can be modified to introduce localized imperfections, increasing charge transfer rates without sacrificing charge mobility.

In line with the importance of crystallinity, also phase composition plays a key role in determining photocatalytic activity. Since both in the case of laboratory-made samples and commercial products, TiO_2 is often obtained as a mixture of polymorphs, the effect that composition purity might exert on the photocatalytic performance of TiO_2 samples is to be considered. Anatase is generally considered to be the most active amongst TiO_2 polymorphs, which has been related to its slightly higher Fermi level, its higher surface density of hydroxyl groups[29,30] and to a lower recombination rate of photogenerated electrons and holes[31]. That said, multiphasic titania can benefit from the combination of different semiconductor phases, following the principle of semiconductor coupling[32,33]. For the photocatalytic efficiency to increase, the recombination of holes and excited electrons generated by UV light must be hindered. To this purpose, semiconductor coupling is generally adopted to create heterojunctions between two, or even three different phases, with the aim of driving photopromoted electrons and/or electron holes to move from the first to the second semiconductor conduction or valence bands, possibly disfavours recombination phenomena. Multiphasic materials could also benefit from an extension of the photoexcitation energy range: as nanometric rutile absorbs in the visible region, making it yellow, it can be combined with anatase to increase its absorption spectrum.

Lastly, good surface hydroxylation is almost always needed to promote dispersibility in water as well as interfacial reactivity. As said before, hydroxyl radicals, which are produced by the combination of photogenerated electron holes in the valence band with surface adsorbed hydroxyl moieties, play the important role of intermediates in the photo-oxidation of adsorbed species.

All that said, the photocatalytic response of TiO_2 photocatalysts appears to rely on a complex combination between the physicochemical features of the material and the nature of the adsorbed molecule reacting. Depending on the nature of the reagent, certain specific structural or morphological parameters might bear more or less importance in affecting photocatalyst activity[34].

II.1.4 Light-induced superhydrophilicity

Among the light-induced behaviours of TiO_2 , there is also the UV-driven modification of wetting properties to form superhydrophilic surfaces. Firstly described in 1997 by Fujishima and co-workers[35], it consists in a drastic increase in surface wettability, both for water and organic liquids, after UV light irradiation. The mechanism behind this phenomenon can be traced back to different phenomena[3]. One of the mechanisms at play regards the decrease of surface contact angle due to the decomposition of fouling organic compounds adsorbed on the TiO_2 surface. As organic contaminants are ubiquitous, TiO_2 surfaces are not exempt from their deposition and consequent hydrophobization. Treatment with UV light induces the photocatalytical degradation of said organic compounds, causing the water contact angle to decrease. When stored in the dark, organic molecules may adsorb on the surface again, increasing the contact angle. While this mechanism is relevant for the superhydrophilization of TiO_2 films, it does not explain the increased affinity for organic liquids as well. The increase in wettability is related to a combination of effects, including the increment of surface hydroxylation caused by a reductive mechanism[36]: as TiO_2 is irradiated with UV light, Ti(IV) cations are reduced to Ti(III) by the simultaneous action of electrons and trapping of holes close to the semiconductor surface. Trapped holes cause the release of oxygen atoms by weakening their bond with titanium, producing oxygen vacancies. When water adsorb to these sites, it dissociates, generating hydroxyl groups: the increase of chemisorbed $-\text{OH}$ favours interactions with water molecules, both through Van der Waals forces and hydrogen bonds, which can thus easily spread across the film surface. The amphiphilic behaviour of treated surfaces can be explained by the formation of alternating localized hydrophilic and hydrophobic regions, measuring few nanometres. Air exposure supposedly re-oxidises the reduced sites and the weakly bound hydroxyl groups can consequently desorb over time from the surface, decreasing wettability. On the same line, a similar oxidative mechanism may lead to the same increase in hydroxylation: according to Sakai et al.[37], oxygen defects would be generated by the trapping of holes, instead of the reduction of Ti(IV) ions, *de facto* leading to the same dissociative adsorption of water molecules previously described. Among the proposed mechanisms, a 2005 study argued that the photoinduced superhydrophilicity of TiO_2 photocatalyst surfaces originates by the desorption of H_2O molecules from the semiconductor surface due to heating from the light sources and the partial elimination of hydrocarbons by photocatalytic decomposition[38]. Since the surface tension of H_2O molecules is quite large compared to that of other liquids, large amounts of H_2O adsorbed on solid surfaces aggregates and forms bulky H_2O clusters, providing free adsorption sites for hydrocarbons. The reduction of the surface tension of H_2O clusters, followed by a decrease of adsorbed H_2O after UV light irradiation, was found to play a major role in the photo-induced phenomenon.

II.2 Applications

Titanium dioxide has historically garnered incredible success due to its advantageous properties, including chemical stability, low cost and high refractive index. At the beginning of the twentieth century, it completely replaced toxic lead oxides as the most diffused white pigment in industry. Considering the gargantuan measure of pigment production worldwide, which currently averages 4 million tons per year, it goes without saying that TiO_2 has deep relations to many aspects of our everyday life, from paints, to plastic and paper, recently including also a series of minor sectors such as textiles, food, leather, cosmetic products, sunscreens and toothpaste. Its high refractive index makes it useful as anti-reflection coating in silicon-based solar cells and many other thin film optical devices. With the advent of nanometric TiO_2 powders and films, its semiconductor nature could be exploited in photocatalysis, opening the way to a long series of technologically advanced applications[36]: it has been used as photocatalyst in environmental remediation[39], in solar cells[40], for the production of hydrogen[41], as gas sensor[42], in anti-corrosion and optical coatings[43,44], in lithium-based batteries[45] and as a component in bone implants[46]. Among the myriad of potential applications of this multifaceted material, the three most relevant to the scope of this thesis are discussed below.

II.2.1 Photocatalytic degradation of organic pollutants

As the use of organic compounds in almost any field has increased, so has their amount in our water reserves and the urge to dispose of them to avoid irremediable pollution has risen. In this sense, photocatalysis is one of the most promising methods for the complete degradation or mineralization of a wide range of water pollutants, as it does not require any potentially toxic oxidant. The use of TiO_2 powders and thin films has been extensively studied and applied for the decomposition of various organic pollutants under both solar and UV light. Among these, compounds encompassing every aspect of our life can be found, such as pesticides, dyes, polymers, phenols and pharmaceuticals[47–51].

Besides lab-scale studies, TiO_2 photocatalysis has shown promising results in wastewater degradation in large scale solar reactors. Pereira et al.[52] studied the photocatalytic degradation of the antibiotic oxytetracycline under solar light in a pilot plant reactor: oxytetracycline and total organic carbon removals were followed as a function of accumulated UV energy entering the reactor. Results showed a 100% oxytetracycline and almost 80% total organic carbon removal after treatment.

Other than water purification, TiO_2 -based devices have also been applied to the treatment of indoor air to remove odours and destroy pathogens and contaminants. Low concentrations of volatile organic compounds (VOCs), such as formaldehyde, toluene, ethanol and acetaldehyde, are emitted from interior furniture and construction materials, while NO_x and SO_x species are generated by vehicles and industrial pollution. Conventional air-purification systems often adopt filter-type components for the cleaning of polluted air. Eventually, pollutants accumulate in the active carbon filters, saturating them and inhibiting their function after a certain period of use. Photocatalytic

air cleaning filters, however, are able to decompose organic substances instead of accumulating them. Ao et al. performed a study on the purification of indoor air through the use of a combined TiO_2 /active carbon filter to degrade NO and toluene[53]: it was found that, while the use of a bare TiO_2 filter removed 83.2% of NO, it generated 12.9% of NO_2 . Using a combined TiO_2 /active carbon filter, NO removal efficiency increased to 97% while the generation of NO_2 decreased to 1.6%. The degradation efficiency of the composite filter compared to the bare TiO_2 filter was even higher when less photoreactive pollutants, such as toluene, were targeted. Filters were both tested in a reaction chamber and installed on a commercial air conditioner.

Another extensively investigated application of TiO_2 concerns the deposition of self-cleaning coatings on surfaces, to impart them the ability of decomposing organic contaminants either by solar exposition or through irradiation with UV light. This way, the need for detergents is significantly reduced, limiting maintenance costs and improving the performance of said surfaces. As of today, many different products have been coated with TiO_2 self-cleaning films, including tiles, window glass, aluminium sheets and cement. A recently published paper investigated the production of flexible polycarbonate surfaces coated with TiO_2 [54]: they fabricated a photocatalytic, superhydrophilic, and self-cleaning coating by incorporating SiO_2 into a titania matrix. After treatment, the surface was optically transparent and was able to remove adsorbed oleic acid after brief UV light irradiation.

II.2.2 Anti-fogging coatings

Glass fogging happens when humid air condenses on a colder surface, such as a mirror or window, forming microscopic water droplets that scatter light, reducing the transparency of the glass. Photo-induced superhydrophilicity can be exploited to prevent the formation of micro droplets by enhancing dramatically the surface wettability. The increased surface adhesion forces overcome water surface tension, leading to the formation of a uniform water film that does not scatter light and is usually thin enough to quickly evaporate. A recent paper by Chemin et al. described the deposition of anti-fogging, self-cleaning anatase/ SiO_2 nanocomposite coatings on polymer substrates[55]. The addition of a SiO_2 precursor during the synthesis of the film caused a steep decrease in water contact angle. As a consequence, coatings exhibited an efficient and durable anti-fogging behaviour. All nanocomposite films were optically transparent and combined three functional properties, namely self-cleaning, anti-fogging and optical transparency, within the same layer. Such coatings could be applied in the mass production of resistant, anti-fogging polymer-based lenses and displays.

II.2.3 Antimicrobial surfaces

The photodegrading action of TiO_2 can also put to use to produce self-sterilizing surfaces. The decomposition of organic compounds can indeed lead to the death of microbial agents, without the need for electrical power nor chemical reagents, relying just on oxygen and light[56]. The combination of three different mechanisms has been proposed to explain the antibacterial behaviour of TiO_2 -based materials[57]: the

generation of highly reactive oxygen species (ROS) at the hand of surface charge carriers, the disruption of cell walls and membranes caused by electrostatic interactions exacerbated by the large surface area of TiO₂ nanoparticles, and the interaction between TiO₂ and intracellular organelles following damage to cell membranes. Among these three potential causes, the production of ROS has been suggested to be the main responsible for the antimicrobial nature of TiO₂ surfaces. Such species, which include $\cdot\text{O}_2^-$, H₂O₂ and $\cdot\text{OH}$, thanks to their oxidative power can destroy cell membrane constituents, damaging the integrity of the membrane and even cross it. The oxidation, and subsequent decomposition of cellular components such as lipids, DNA strands and proteins can obviously lead to function loss and ultimately to cellular death (apoptosis)[57].

For these reasons, many efforts have been devoted to the study and application of TiO₂-based materials and coatings as efficient, low-maintenance antimicrobial surfaces, to be used in environments which require high sanitary standards[58,59]. For example, Arellano et al.[60] explored the antibacterial efficacy of Fe-doped TiO₂ films, deposited on a sodium glass substrate, against *E. coli* under visible light. Results showed that TiO₂ films containing 5% of Fe eliminated *E. coli* completely after 60 minutes of light exposure. The authors explained these results on the basis of the large surface area available for bacterial adhesion together with the lower band gap provided by the doping with Fe, which enhanced the photocatalytic response under visible light. Another study focused on the preparation of flexible, antibacterial TiO₂ films, prepared by mixing titania with polyethylene, indium oxide and polyester[61]. Bacterial apoptosis was supposedly induced by the interactions between cell membranes and TiO₂ nanoparticles, which stripped membrane constituents off the pathogen. In this case, TiO₂-polyester surfaces were shown to inactivate bacteria even in the dark, an interesting result not often addressed in bacterial inactivation studies, which generally focus on TiO₂ photocatalysis. Sunada and co-workers studied the photo-induced cytotoxic activity of TiO₂ films deposited on silica-coated soda lime glass against *E. coli*[62]. *In vitro* experiments on the survival of *E. coli* cells deposited on illuminated TiO₂ films suggested a two-step decay mechanism: the first step of the apoptotic process is the disordering of the outer membrane of bacteria cells by irradiated TiO₂ particles. This process proved necessary for the penetration of reactive species produced by photocatalysis up to the inner membrane. The second step of the process is the disruption of the inner cytoplasmic membrane by ROS oxidation. In a recent paper, Pleskova et al. described a new way of enhancing the antibacterial action of TiO₂ films through the formation of nanopores on the surface by means of four-beam high-power laser irradiation[63]. Such surfaces showed significantly higher antibacterial activity compared to conventional TiO₂ surfaces. This enhancement was reportedly caused by the increased surface area of nanoporous surfaces, allowing thus for increased contact between bacteria and the semiconductor surface. At the same time, the larger surface area was linked to an increase in ROS production. The authors suggest that this phenomenon could be caused by localized changes in the crystal structure of TiO₂ films, from amorphous to more crystalline, following the laser treatment, leading to slower recombination rates for electron-hole pairs. Moreover, as the oxidation of microorganisms mostly occurs in direct contact with the semiconductor surface, due to the short lifetime and migration radius of most ROS, it was proposed that the higher

bactericidal effect could also be caused by nanopores trapping bacteria, thus favouring adhesion.

Given the extremely vast horizon of TiO₂ applications, it is worrying to read that recent studies have suggested that TiO₂ might induce DNA damage and genetic instability[25]. Although epidemiologic studies on industry workers, investigating the exposure to pigment-grade TiO₂, were not able to detect a significant association between occupational contact with titanium dioxide and lung cancer[64], the large diffusion of nanometric TiO₂ in commercial products could still present issues for human health. In light of these concerns, the TiO₂-based devices presented in this thesis show great potential thanks to their mechanical stability and absence of volatile powders. The next chapters will in fact explore the synthesis and functionalization of TiO₂ films with the purpose of applying them for a wide range of applications. While Chapters 4 and 5 represent a more didactic take on the ever-growing subject of superhydrophobic and superhydrophilic surfaces, Chapters 6 and 7 will tackle specific applicative issues, such as sensor fouling and antimicrobial surfaces, through an approach based on highly porous TiO₂ films.

References

- [1] A. FUJISHIMA, K. HONDA, Electrochemical Photolysis of Water at a Semiconductor Electrode, *Nature*. 238 (1972) 37–38. doi:10.1038/238037a0.
- [2] A. FUJISHIMA, X. ZHANG, D. TRYK, TiO₂ photocatalysis and related surface phenomena, *Surf. Sci. Rep.* 63 (2008) 515–582. doi:10.1016/j.surfrep.2008.10.001.
- [3] K. Liu, M. Cao, A. Fujishima, L. Jiang, Bio-Inspired Titanium Dioxide Materials with Special Wettability and Their Applications, *Chem. Rev.* 114 (2014) 10044–10094. doi:10.1021/cr4006796.
- [4] D.A.H. Hanaor, C.C. Sorrell, Review of the anatase to rutile phase transformation, *J. Mater. Sci.* 46 (2011) 855–874. doi:10.1007/s10853-010-5113-0.
- [5] Y. Al-Khatatbeh, K.K.M. Lee, B. Kiefer, High-pressure behavior of TiO₂ as determined by experiment and theory, *Phys. Rev. B.* 79 (2009) 134114. doi:10.1103/PhysRevB.79.134114.
- [6] D.T. Cromer, K. Herrington, The Structures of Anatase and Rutile, *J. Am. Chem. Soc.* 77 (1955) 4708–4709. doi:10.1021/ja01623a004.
- [7] Rutile mineral data, (n.d.). <https://www.mindat.org/min-3486.html> (accessed February 22, 2021).
- [8] Anatase mineral data, (n.d.). <https://www.mindat.org/min-213.html> (accessed February 22, 2021).
- [9] T. Zhu, S.-P. Gao, The Stability, Electronic Structure, and Optical Property of TiO₂ Polymorphs, *J. Phys. Chem. C.* 118 (2014) 11385–11396. doi:10.1021/jp412462m.
- [10] Brookite mineral data, (n.d.). <https://www.mindat.org/min-787.html> (accessed February 22, 2021).

- [11] Q. Wang, M. Watanabe, K. Edalati, Visible-Light Photocurrent in Nanostructured High-Pressure TiO₂ -II (Columbite) Phase, *J. Phys. Chem. C*. 124 (2020) 13930–13935. doi:10.1021/acs.jpcc.0c03923.
- [12] S. Patra, C. Davoisne, H. Bouyanfif, D. Foix, F. Sauvage, Phase stability frustration on ultra-nanosized anatase TiO₂, *Sci. Rep.* 5 (2015) 10928. doi:10.1038/srep10928.
- [13] H. Zhang, J.F. Banfield, Thermodynamic analysis of phase stability of nanocrystalline titania, *J. Mater. Chem.* 8 (1998) 2073–2076. doi:10.1039/a802619j.
- [14] W. Li, C. Ni, H. Lin, C.P. Huang, S.I. Shah, Size dependence of thermal stability of TiO₂ nanoparticles, *J. Appl. Phys.* 96 (2004) 6663–6668. doi:10.1063/1.1807520.
- [15] L. Andronic, D. Andrasi, A. Enesca, M. Visa, A. Duta, The influence of titanium dioxide phase composition on dyes photocatalysis, *J. Sol-Gel Sci. Technol.* 58 (2011) 201–208. doi:10.1007/s10971-010-2378-3.
- [16] S. Zhu, D. Wang, Photocatalysis: Basic Principles, Diverse Forms of Implementations and Emerging Scientific Opportunities, *Adv. Energy Mater.* 7 (2017) 1700841. doi:10.1002/aenm.201700841.
- [17] W. Choi, J.Y. Choi, H. Song, Regulation of electron-hole recombination kinetics on uniform metal-semiconductor nanostructures for photocatalytic hydrogen evolution, *APL Mater.* 7 (2019) 100702. doi:10.1063/1.5099666.
- [18] H. Yan, X. Wang, M. Yao, X. Yao, Band structure design of semiconductors for enhanced photocatalytic activity: The case of TiO₂, *Prog. Nat. Sci. Mater. Int.* 23 (2013) 402–407. doi:10.1016/j.pnsc.2013.06.002.
- [19] C. Xiang, K.M. Papadantonakis, N.S. Lewis, Principles and implementations of electrolysis systems for water splitting, *Mater. Horizons*. 3 (2016) 169–173. doi:10.1039/C6MH00016A.
- [20] U.G. Akpan, B.H. Hameed, Parameters affecting the photocatalytic degradation of dyes using TiO₂-based photocatalysts: A review, *J. Hazard. Mater.* 170 (2009) 520–529. doi:10.1016/j.jhazmat.2009.05.039.
- [21] W.-K. Jo, T.S. Natarajan, Influence of TiO₂ morphology on the photocatalytic efficiency of direct Z-scheme g-C₃N₄/TiO₂ photocatalysts for isoniazid degradation, *Chem. Eng. J.* 281 (2015) 549–565. doi:10.1016/j.cej.2015.06.120.
- [22] T.L. Thompson, J.T. Yates, TiO₂-based Photocatalysis: Surface Defects, Oxygen and Charge Transfer, *Top. Catal.* 35 (2005) 197–210. doi:10.1007/s11244-005-3825-1.
- [23] A.L. Stroyuk, A.I. Kryukov, S.Y. Kuchmii, V.D. Pokhodenko, Quantum Size Effects in Semiconductor Photocatalysis, *Theor. Exp. Chem.* 41 (2005) 207–228. doi:10.1007/s11237-005-0042-8.
- [24] M. Anpo, T. Shima, S. Kodama, Y. Kubokawa, Photocatalytic hydrogenation of propyne with water on small-particle titania: size quantization effects and reaction intermediates, *J. Phys. Chem.* 91 (1987) 4305–4310. doi:10.1021/j100300a021.
- [25] C. Kormann, D.W. Bahnemann, M.R. Hoffmann, Preparation and characterization of quantum-size titanium dioxide, *J. Phys. Chem.* 92 (1988) 5196–5201. doi:10.1021/j100329a027.
- [26] H. LIN, C. HUANG, W. LI, C. NI, S. SHAH, Y. TSENG, Size dependency of

nanocrystalline TiO₂ on its optical property and photocatalytic reactivity exemplified by 2-chlorophenol, *Appl. Catal. B Environ.* 68 (2006) 1–11. doi:10.1016/j.apcatb.2006.07.018.

- [27] M. Bellardita, A. Di Paola, B. Megna, L. Palmisano, Determination of the crystallinity of TiO₂ photocatalysts, *J. Photochem. Photobiol. A Chem.* 367 (2018) 312–320. doi:10.1016/j.jphotochem.2018.08.042.
- [28] H. Zhao, F. Pan, Y. Li, A review on the effects of TiO₂ surface point defects on CO₂ photoreduction with H₂O, *J. Mater.* 3 (2017) 17–32. doi:10.1016/j.jmat.2016.12.001.
- [29] R.I. Bickley, T. Gonzalez-Carreno, J.S. Lees, L. Palmisano, R.J.D. Tilley, A structural investigation of titanium dioxide photocatalysts, *J. Solid State Chem.* 92 (1991) 178–190. doi:10.1016/0022-4596(91)90255-G.
- [30] H. Gerischer, A. Heller, Photocatalytic Oxidation of Organic Molecules at TiO₂ Particles by Sunlight in Aerated Water, *J. Electrochem. Soc.* 139 (1992) 113–118. doi:10.1149/1.2069154.
- [31] K. Yanagisawa, J. Ovenstone, Crystallization of Anatase from Amorphous Titania Using the Hydrothermal Technique: Effects of Starting Material and Temperature, *J. Phys. Chem. B.* 103 (1999) 7781–7787. doi:10.1021/jp990521c.
- [32] D.C. Hurum, A.G. Agrios, K.A. Gray, T. Rajh, M.C. Thurnauer, Explaining the Enhanced Photocatalytic Activity of Degussa P25 Mixed-Phase TiO₂ Using EPR, *J. Phys. Chem. B.* 107 (2003) 4545–4549. doi:10.1021/jp0273934.
- [33] X. Jiang, M. Manawan, T. Feng, R. Qian, T. Zhao, G. Zhou, F. Kong, Q. Wang, S. Dai, J.H. Pan, Anatase and rutile in evonik aerioxide P25: Heterojunctioned or individual nanoparticles?, *Catal. Today.* 300 (2018) 12–17. doi:10.1016/j.cattod.2017.06.010.
- [34] S. Ardizzzone, C.L. Bianchi, G. Cappelletti, S. Gialanella, C. Pirola, V. Ragaini, Tailored Anatase/Brookite Nanocrystalline TiO₂. The Optimal Particle Features for Liquid- and Gas-Phase Photocatalytic Reactions, *J. Phys. Chem. C.* 111 (2007) 13222–13231. doi:10.1021/jp0741096.
- [35] R. Wang, K. Hashimoto, A. Fujishima, M. Chikuni, E. Kojima, A. Kitamura, M. Shimohigoshi, T. Watanabe, Light-induced amphiphilic surfaces, *Nature.* 388 (1997) 431–432. doi:10.1038/41233.
- [36] O. Carp, C.L. Huisman, A. Reller, Photoinduced reactivity of titanium dioxide, *Prog. Solid State Chem.* 32 (2004) 33–177. doi:10.1016/j.progsolidstchem.2004.08.001.
- [37] N. Sakai, A. Fujishima, T. Watanabe, K. Hashimoto, Enhancement of the Photoinduced Hydrophilic Conversion Rate of TiO₂ Film Electrode Surfaces by Anodic Polarization, *J. Phys. Chem. B.* 105 (2001) 3023–3026. doi:10.1021/jp003212r.
- [38] M. Takeuchi, K. Sakamoto, G. Martra, S. Coluccia, M. Anpo, Mechanism of Photoinduced Superhydrophilicity on the TiO₂ Photocatalyst Surface, *J. Phys. Chem. B.* 109 (2005) 15422–15428. doi:10.1021/jp058075i.
- [39] R. Daghrir, P. Drogui, D. Robert, Modified TiO₂ For Environmental Photocatalytic Applications: A Review, *Ind. Eng. Chem. Res.* 52 (2013) 3581–3599. doi:10.1021/ie303468t.
- [40] D.Y. Rahman, M. Rokhmat, E. Yuliza, E. Sustini, M. Abdullah, New design of potentially low-cost solar cells using TiO₂/graphite composite as photon absorber, *Int.*

J. Energy Environ. Eng. 7 (2016) 289–296. doi:10.1007/s40095-016-0213-5.

- [41] B. Wang, S. Shen, S.S. Mao, Black TiO₂ for solar hydrogen conversion, J. Mater. 3 (2017) 96–111. doi:10.1016/j.jmat.2017.02.001.
- [42] B. Karunagaran, P. Uthirakumar, S.J. Chung, S. Velumani, E.-K. Suh, TiO₂ thin film gas sensor for monitoring ammonia, Mater. Charact. 58 (2007) 680–684. doi:10.1016/j.matchar.2006.11.007.
- [43] S. Li, J. Fu, Improvement in corrosion protection properties of TiO₂ coatings by chromium doping, Corros. Sci. 68 (2013) 101–110. doi:10.1016/j.corsci.2012.10.040.
- [44] C. Euvananont, C. Junin, K. Inpor, P. Limthongkul, C. Thanachayanont, TiO₂ optical coating layers for self-cleaning applications, Ceram. Int. 34 (2008) 1067–1071. doi:10.1016/j.ceramint.2007.09.043.
- [45] X. Yan, Z. Wang, M. He, Z. Hou, T. Xia, G. Liu, X. Chen, TiO₂ Nanomaterials as Anode Materials for Lithium-Ion Rechargeable Batteries, Energy Technol. 3 (2015) 801–814. doi:10.1002/ente.201500039.
- [46] T.-K. Ahn, D.H. Lee, T. Kim, G. chol Jang, S. Choi, J.B. Oh, G. Ye, S. Lee, Modification of Titanium Implant and Titanium Dioxide for Bone Tissue Engineering, Adv. Exp. Med. Biol. (2018) 355–368. doi:10.1007/978-981-13-0947-2_19.
- [47] M. Pelaez, P. Falaras, V. Likodimos, A.G. Kontos, A.A. de la Cruz, K. O’shea, D.D. Dionysiou, Synthesis, structural characterization and evaluation of sol–gel-based NF-TiO₂ films with visible light-photoactivation for the removal of microcystin-LR☆, Appl. Catal. B Environ. 99 (2010) 378–387. doi:10.1016/j.apcatb.2010.06.017.
- [48] S. Gelover, P. Mondragón, A. Jiménez, Titanium dioxide sol–gel deposited over glass and its application as a photocatalyst for water decontamination, J. Photochem. Photobiol. A Chem. 165 (2004) 241–246. doi:10.1016/j.jphotochem.2004.03.023.
- [49] S. Ahmed, M.G. Rasul, R. Brown, M.A. Hashib, Influence of parameters on the heterogeneous photocatalytic degradation of pesticides and phenolic contaminants in wastewater: A short review, J. Environ. Manage. 92 (2011) 311–330. doi:10.1016/j.jenvman.2010.08.028.
- [50] S.M. Gupta, M. Tripathi, A review of TiO₂ nanoparticles, Chinese Sci. Bull. 56 (2011) 1639–1657. doi:10.1007/s11434-011-4476-1.
- [51] C.W.H. Dunnill, Z.A. Aiken, J. Pratten, M. Wilson, D.J. Morgan, I.P. Parkin, Enhanced photocatalytic activity under visible light in N-doped TiO₂ thin films produced by APCVD preparations using t-butylamine as a nitrogen source and their potential for antibacterial films, J. Photochem. Photobiol. A Chem. 207 (2009) 244–253. doi:10.1016/j.jphotochem.2009.07.024.
- [52] J.H.O.S. Pereira, V.J.P. Vilar, M.T. Borges, O. González, S. Esplugas, R.A.R. Boaventura, Photocatalytic degradation of oxytetracycline using TiO₂ under natural and simulated solar radiation, Sol. Energy. 85 (2011) 2732–2740. doi:10.1016/j.solener.2011.08.012.
- [53] C.H. Ao, S.C. Lee, Indoor air purification by photocatalyst TiO₂ immobilized on an activated carbon filter installed in an air cleaner, Chem. Eng. Sci. 60 (2005) 103–109. doi:10.1016/j.ces.2004.01.073.
- [54] T. Adachi, S.S. Latthe, S.W. Gosavi, N. Roy, N. Suzuki, H. Ikari, K. Kato, K.

Katsumata, K. Nakata, M. Furudate, T. Inoue, T. Kondo, M. Yuasa, A. Fujishima, C. Terashima, Photocatalytic, superhydrophilic, self-cleaning TiO₂ coating on cheap, light-weight, flexible polycarbonate substrates, *Appl. Surf. Sci.* 458 (2018) 917–923. doi:10.1016/j.apsusc.2018.07.172.

- [55] J.-B. Chemin, S. Bulou, K. Baba, C. Fontaine, T. Sindzingre, N.D. Boscher, P. Choquet, Transparent anti-fogging and self-cleaning TiO₂/SiO₂ thin films on polymer substrates using atmospheric plasma, *Sci. Rep.* 8 (2018) 9603. doi:10.1038/s41598-018-27526-7.
- [56] Pant, Park, Park, Recent Advances in TiO₂ Films Prepared by Sol-gel Methods for Photocatalytic Degradation of Organic Pollutants and Antibacterial Activities, *Coatings*. 9 (2019) 613. doi:10.3390/coatings9100613.
- [57] J. Hou, L. Wang, C. Wang, S. Zhang, H. Liu, S. Li, X. Wang, Toxicity and mechanisms of action of titanium dioxide nanoparticles in living organisms, *J. Environ. Sci.* 75 (2019) 40–53. doi:10.1016/j.jes.2018.06.010.
- [58] C.-J. Chung, H.-I. Lin, H.-K. Tsou, Z.-Y. Shi, J.-L. He, An antimicrobial TiO₂ coating for reducing hospital-acquired infection, *J. Biomed. Mater. Res. Part B Appl. Biomater.* 85B (2008) 220–224. doi:10.1002/jbm.b.30939.
- [59] S.P. Krumdieck, R. Boichot, R. Gorthy, J.G. Land, S. Lay, A.J. Gardecka, M.I.J. Polson, A. Wasa, J.E. Aitken, J.A. Heinemann, G. Renou, G. Berthomé, F. Charlot, T. Encinas, M. Braccini, C.M. Bishop, Nanostructured TiO₂ anatase-rutile-carbon solid coating with visible light antimicrobial activity, *Sci. Rep.* 9 (2019) 1883. doi:10.1038/s41598-018-38291-y.
- [60] U. Arellano, M. Asomoza, F. Ramírez, Antimicrobial activity of Fe–TiO₂ thin film photocatalysts, *J. Photochem. Photobiol. A Chem.* 222 (2011) 159–165. doi:10.1016/j.jphotochem.2011.05.016.
- [61] J. Kiwi, S. Rtimi, R. Sanjines, C. Pulgarin, TiO₂ and TiO₂ -Doped Films Able to Kill Bacteria by Contact: New Evidence for the Dynamics of Bacterial Inactivation in the Dark and under Light Irradiation, *Int. J. Photoenergy*. 2014 (2014) 1–17. doi:10.1155/2014/785037.
- [62] K. Sunada, T. Watanabe, K. Hashimoto, Studies on photokilling of bacteria on TiO₂ thin film, *J. Photochem. Photobiol. A Chem.* 156 (2003) 227–233. doi:10.1016/S1010-6030(02)00434-3.
- [63] S.N. Pleskova, I.S. Golubeva, Y.K. Verevkin, Bactericidal activity of titanium dioxide ultraviolet-induced films, *Mater. Sci. Eng. C*. 59 (2016) 807–817. doi:10.1016/j.msec.2015.10.021.
- [64] P. Boffetta, V. Gaborieau, L. Nadon, M.-E. Parent, E. Weiderpass, J. Siemiatycki, Exposure to titanium dioxide and risk of lung cancer in a population-based study from Montreal, *Scand. J. Work. Environ. Health*. 27 (2001) 227–232. doi:10.5271/sjweh.609.

CHAPTER 4

Bouncing droplets: a hands-on activity to demonstrate the properties and applications of superhydrophobic surface coatings

Besides the more conventional applications of TiO_2 photocatalysts for environmental remediation and energy conversion[1,2], researchers have more recently started to explore the application of TiO_2 materials to the preparation of surfaces with tailored wetting features[3]. This exciting research field has led to the development of materials with enticing surface properties, such as superhydrophobic and superhydrophilic coatings, which have found application as antifogging mirrors[4], self-cleaning ceramics[5], anti-stain fabrics[6], anti-corrosion coatings[7], liquid transportation and separation devices[8]. These materials have widespread potential application in everyday life and are based on clearly appreciable and immediate phenomena.

Interestingly, the application of photocatalysis for the tailoring of wetting phenomena is well-suited to provide engaging activities for students of all levels, which can introduce the general concepts of photocatalysis in a more tangible and compelling way than conventional experiments such as pollutant degradation tests[9–12]. Moreover, only few works have proposed teaching laboratory activities on the modification of wetting properties of oxide materials by chemical[13–15] or physical[16] means. Physical chemistry courses in Chemistry and Materials Science curricula often overlook these concepts and even more rarely Bachelor's and Master's students take part in laboratory activities providing a direct approach to these concepts.

In this respect, I was directly involved in outreach and teaching activities regarding the physical chemistry of interfaces. The next two Chapters will illustrate two original, hands-on activities that I contributed to develop, regarding the modification of the surface properties of TiO_2 -based films to demonstrate basic concepts of wetting and adhesion as well as the application of photocatalysis to the modification of surface properties. Experimental procedures were designed to serve as a learning activity for high school and undergraduate students, with a marked focus on the practical demonstration of discussed topics. The results reported in these Chapters were thus published on the Journal of Chemical education.

The first activity, that will be presented in this Chapter, illustrates the modification of the TiO_2 surface features from their inherent hydrophilic behavior to water repellency. Superhydrophobicity, which consists in the complete repulsion of water by a surface, is a phenomenon often observed in nature, such as in lotus leaves, rose petals, butterfly wings, gecko feet and other surfaces found in a variety of other animals and plants[17]. In natural surfaces, superhydrophobicity is achieved via the synergy of multiscale texture and surface chemical composition[18]. Much effort has been devoted to

mimicking natural superhydrophobic surfaces owing to the numerous potential applications of materials with controlled wetting properties: self-cleaning and anti-stain materials, liquid-transport and drag-reduction systems, anti-corrosion and anti-icing surfaces, just to name a few[3]. In the last two decades, the field of wetting modification has experienced tremendous breakthroughs both in terms of more accurate mimics of natural materials[18,19] as well as in the development of surfaces with unprecedented wetting features[20–22]. Drawing inspiration from nature, surfaces with robust self-cleaning superhydrophobicity could be designed[23,24] and, thanks to a firmer theoretical understanding of wetting phenomena, innovative properties could be achieved, such as high adhesive superhydrophobicity[25], anisotropic superhydrophobicity[26], switchable superhydrophobicity[27,28], superomniphobicity[29] and superhydrophobicity from inherently hydrophilic materials[30,31].

Despite the numerous real-life applications and exciting research prospects, the literature offers only a few laboratory activities on surface wetting modification[13,32–36]. Moreover, most of them deal with simple hydrophilic/hydrophobic surfaces. Only very few reports offer laboratory activities discussing the preparation of superhydrophobic surfaces[14,37,38]. However, these experiences require long waiting times (from several hours to 2 days) and the preparation and characterization steps often require uncommon equipment for teaching laboratories, like UV/ozone cleaners or spin coaters, and expensive instrumentation, such as contact angle measurement instruments and electron microscopes.

In this Chapter, a simple and fast hands-on activity for the preparation of superhydrophobic coatings based on the deposition of surface functionalized oxide powders was proposed. Much emphasis was given to the observation of the superhydrophobic coating properties, highlighting the potential applications in everyday life: from rolling-off and bouncing droplets, to liquid transport capabilities, self-cleaning and anti-stain features.

EXPERIMENTAL OVERVIEW

Wetting describes the interface phenomena occurring between a solid and a liquid and it is usually expressed in terms of contact angle, θ . A first description of the contact angle of a liquid droplet on a solid surface was given by Young in 1805[39], who related wetting to the liquid surface tension and solid surface free energy. Young's theoretical model referred to ideally flat and homogeneous surfaces (Figure 4.1A), hence it did not suit well real solids, which are characterized by surface roughness and composition heterogeneity. As a matter of fact, surface texture has a crucial role on the wetting properties of real surfaces. Two theoretical interpretations of the solid-liquid interaction that explicitly take into account surface roughness, were later proposed by Wenzel[40] and by Cassie and Baxter[41]. Wenzel model assumes that, when a droplet comes into contact with a rough surface, the solid-liquid contact line follows the material topological variations (Figure 4.1B). Therefore, in accordance with this model, surface roughness increases the solid-liquid interface, enhancing the natural wetting properties of the material: rough hydrophilic surfaces become more hydrophilic than their flat counterparts, whereas hydrophobic ones show increased hydrophobicity. However,

when it comes to hydrophobic systems with a high degree of surface roughness and, in particular, solids where micro and nanoscale textures coexist (such as micrometric bumps covered by nanostructures, as in lotus leaves), a high degree of water repellency is observed. The so called superhydrophobic behavior, characterized by water contact angles higher than 150° and very low contact angle hysteresis (*i.e.*, low adhesive behavior), cannot be explained by the Wenzel theory. When a hydrophobic surface becomes very rough, the liquid does not follow the material profile as hydrophobic materials have a higher surface energy wet than dry. Conversely, the droplet will trap air pockets inside the solid texture, sitting on both solid and air (Figure 4.1C). This behavior is called a Cassie-Baxter state. As the liquid contacts the solid only at the top of the surface asperities, droplets in a Cassie-Baxter state show very limited interaction with the solid and hence very low adhesion (non-sticking drops); this behavior is responsible for the self-cleaning and anti-stain properties of superhydrophobic surfaces[42,43].

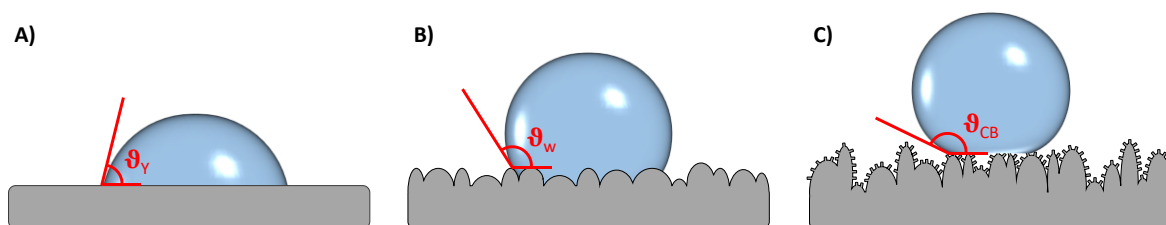


Figure 4.1 - Wetting of a solid surface by a liquid droplet: Young(A), Wenzel (B) and Cassie-Baxter (C) models.

Even though current theoretical understanding has moved past these classical models[44], surface texture has been confirmed to be the most crucial parameter imparting superhydrophobicity. Countless strategies to produce superhydrophobic coatings have been proposed, differing for the adopted materials and strategies for surface texture tailoring. In the present activity, metal oxide powders are employed to provide a multiscale surface roughness[45]; their intrinsically hydrophilic surface, due to the presence of hydroxyl groups, is turned into a hydrophobic one by grafting with alkylsilanes, which form stable siloxane bonds with the surface[46,47] (Figure 4.2A). Superhydrophobic coatings can then be easily prepared by depositing the functionalized particles on a substrate (e.g., glass slides, Petri dishes) by a simple drop-casting deposition (Figure 4.2B).

Although different oxides can be used, in this study a stable and low-cost TiO_2 powder was adopted. In this way, this laboratory procedure can be easily integrated with the hands-on activity reported in the next Chapter on the photocatalytic lithography of superhydrophobic surfaces to achieve superhydrophobic/superhydrophilic wetting contrast.

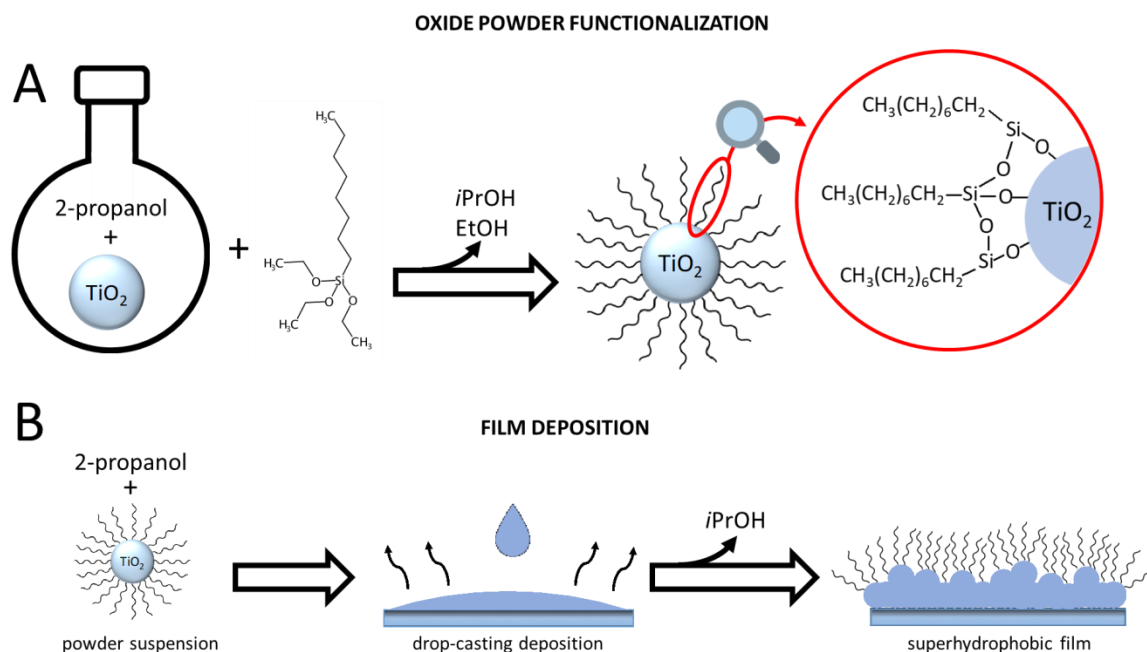


Figure 4.2 - Schematic representation of the superhydrophobic coating preparation: hydrophobic nanoparticles were prepared by impregnating commercial anatase particles with triethoxy(octyl)silane (A); said particles were then deposited by drop-casting a 2-propanol suspension on a glass slide and letting the solvent evaporate, producing a superhydrophobic film (B).

4.1 Experimental procedures

4.1.1 Materials and Instrumentation

Anatase-rutile titanium (IV) oxide (*ca.* $50 \text{ m}^2 \text{ g}^{-1}$), triethoxy(octyl)silane, 2-propanol, acetone, methyl orange and PTFE sheets were purchased from Sigma Aldrich; water passed through a MilliQ apparatus was adopted for the preparation of dye solutions. An ultrasonic bath was used for suspension preparation. Surface functionalization was carried out using a rotary evaporator. Superhydrophobic coatings were deposited on soda lime glass slides and glass Petri dishes.

4.1.2 Functionalization of TiO_2 powder with triethoxy(octyl)silane

In a 100 mL one-neck flask, 0.5 g of oxide was suspended in 50 mL of 2-propanol and then sonicated for 5 min to improve dispersion. Five drops of triethoxy(octyl)silane were added with a Pasteur pipette and the flask was then connected to a rotary evaporator: at ambient pressure, the rotation speed was set to 200 rpm and the bath temperature was progressively increased from room temperature to 50°C within 30 min. At 50°C , the flask was connected to the vacuum line to remove the solvent (approximately 30 min). The dry powders were then collected in a test tube. The successful functionalization of the oxide was assessed by comparing its dispersibility in water with that of the pristine powder.

4.1.3 Drop-casting deposition of superhydrophobic coatings

Substrates (3 glass slides) were cleaned with acetone, rinsed with water, then dried. Functionalized powders were resuspended in 2-propanol (0.05 g in 5 mL) and deposited by drop casting on one of the glass substrates. Solvent was removed by evaporation within a fume hood: the solvent removal can be speeded up by fanning. Reference coatings were prepared by depositing pristine TiO_2 powder on another glass slide according to the same procedure.

4.1.4 Testing of anti-stain properties

The anti-stain properties of superhydrophobic coatings were tested against different kinds of aqueous solutions, such as dye solutions or wine. The liquid was poured with a Pasteur pipette on the coated substrate, which was kept at tilt by placing a glass rod underneath or simply by holding it. As a reference, anti-stain tests were performed also on uncoated glass, PTFE foil and a pristine oxide coated glass slide.

4.1.5 Testing of self-cleaning properties

In order to test the self-cleaning capability of the superhydrophobic coating, graphite powder (prepared by grinding a pencil tip) or other colored powder (*e.g.*, methyl orange) was deposited on the coated glass slide. Then, water was dripped using a Pasteur pipette on the tilted substrate to remove the colored powder with the minimum amount of water. The experiment was repeated on the other provided supports (uncoated glass slide, PTFE foil, and glass coated with pristine oxide) to compare their different behavior.

4.1.6 Liquid transport tests

A suspension of magnetic particles (iron filings) in water (*ca.* 5 mg mL^{-1}) was prepared. A drop of the magnetic particle suspension was gently deposited on the superhydrophobic coating. The liquid droplet was then moved across the surface, controlled by a small magnet placed under the substrate.

4.2 Results and discussion

4.2.1 Powder functionalization and film deposition

The oxide powder was functionalized with triethoxy(octyl)silane by reaction between the hydroxyl groups at the TiO_2 surface and the siloxane head-group. The success of the functionalization procedure can be checked by a simple dispersibility test: while pristine oxide powders readily disperse in water forming a white slurry thanks to their hydrophilicity, surface functionalized particles float even after vigorous agitation (Figure 4.3).

Superhydrophobic coatings were prepared by drop casting deposition of the functionalized powder on clean glass slides: this film preparation method is quick, simple and does not require any expensive equipment. The coating roughness imparted

by the oxide particles, together with the hydrophobicity of the alkylsilane hydrocarbon chains, gave rise to a Cassie-Baxter behavior of the hybrid coating. Resulting films appeared to be almost non-transparent, being prepared from a suspension of relatively large, thus light-scattering, colloids, but showed a relatively good scratch resistance, as reported in Figure 4.4.

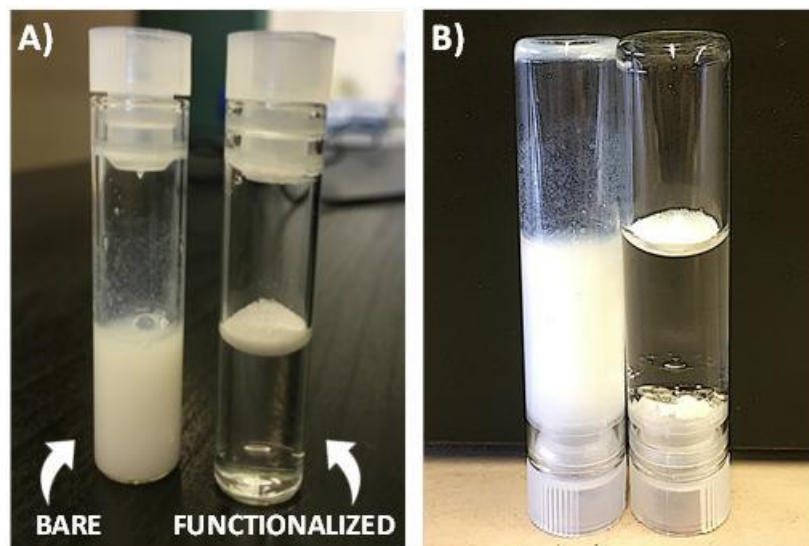


Figure 4.3 - Dispersibility of bare (left vial) and functionalized (right vial) oxide powders (A); flipping or vigorously agitating the vials does not improve the dispersibility of the functionalized particles (B).

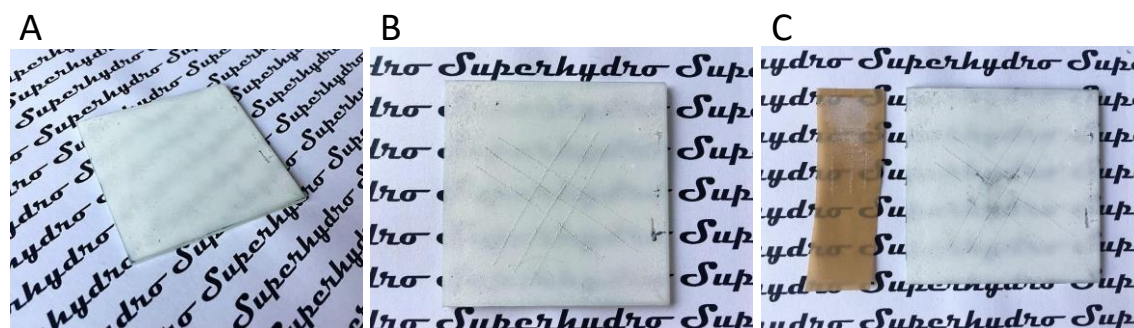


Figure 4.4 - Transparency assessment of prepared films (A); Scratch-resistance test on superhydrophobic films after cross-cutting the layer with a razor blade (B) and pressing and removing an adhesive tape (C), to evaluate coating adhesion.

4.2.2 Application tests

The superhydrophobic coating properties were tested towards different kinds of applications: self-cleaning, anti-stain and liquid transport. For the sake of comparison, tests were always performed also on reference substrates: uncoated glass slides, PTFE foil and pristine oxide films as model surfaces displaying hydrophilic, hydrophobic and superhydrophilic behavior, respectively.

The anti-stain properties of the superhydrophobic coatings were demonstrated by pouring colored aqueous solutions, such as wine or dye solution, on the surface: the liquid droplets were repelled by the surface and rolled off the film without sullyng it (Figure 4.5A). The contrast with the reference surfaces is sharp, especially with the uncoated oxide coating, which readily absorbed the dye solution/wine producing large stains (Figure 4.6).

Moreover, the self-cleaning ability of the functionalized oxide coating was evaluated by covering the surface with graphite or methyl orange powders: by dripping water on the slightly tilted surface, droplets rolling off the surface easily remove dirt particles deposited on it (Figure 4.5B). No similar effects could be observed on the reference samples, even on the highly hydrophobic PTFE foil.

Finally, liquid transport tests were performed using a magnetic particle suspension and a magnet: students were able to drive at will a liquid droplet simply by moving the magnet under the film (Figure 4.5C). The liquid movement can be highlighted by covering the surface with a colored powder (*e.g.*, dye powder as in Figure 4.5C). With respect to hydrophobic surfaces, liquid transport on superhydrophobic surfaces takes place with no loss of liquid enabling the transport even of very small droplets.

As previously mentioned, the prepared superhydrophobic surfaces can also be adopted to perform photocatalytic lithography according to the procedure reported in the next Chapter.

The superhydrophobic features can be conveniently displayed also by depositing the hybrid coating on a glass Petri dish: with respect to glass slides, the enclosed walls of the Petri dish make easier to demonstrate the dynamic properties of the non-sticking droplets (rolling-off and bouncing of droplets of water and aqueous solutions). This latter approach is very engaging and it is well suited also for demonstrations at science fairs and other events.

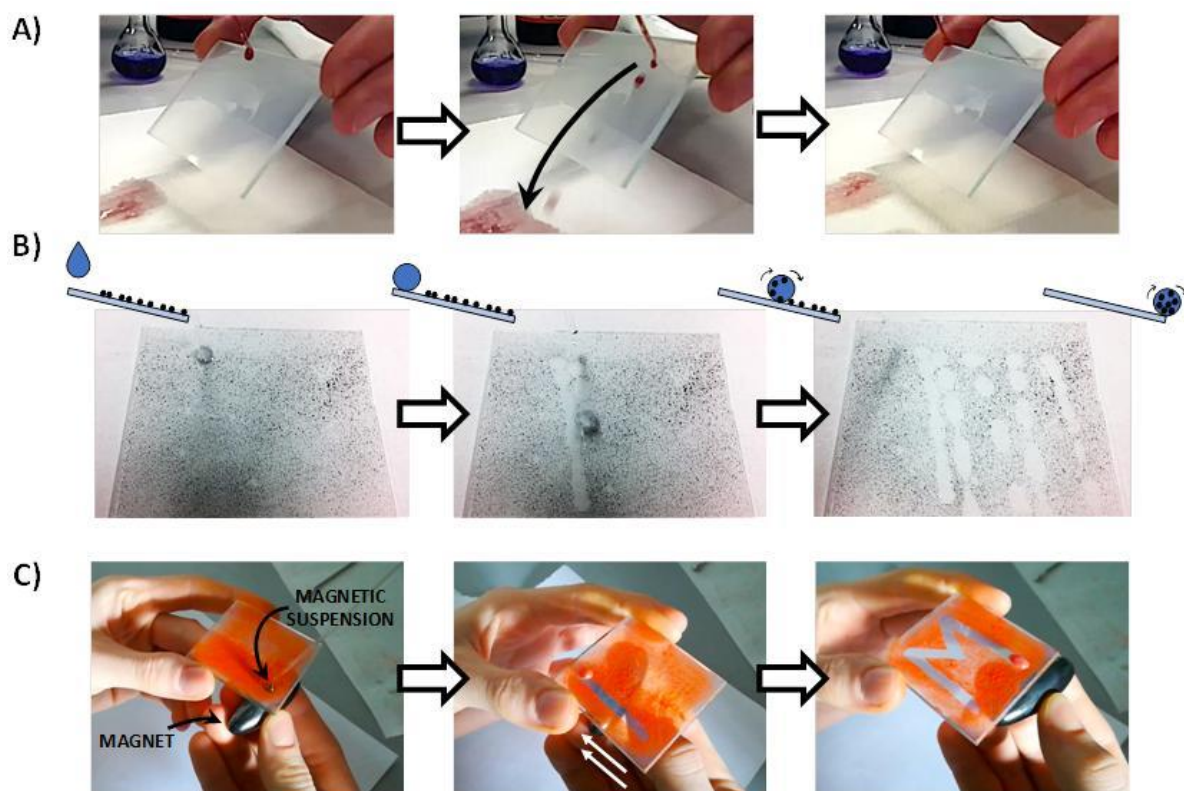


Figure 4.5 - (A) Anti-stain properties: wine is poured on the superhydrophobic coating, which repels it leaving a clean surface. (B) Self-cleaning properties: the hybrid coating covered by graphite powder is easily cleaned by dripping water onto its surface; inset: schematic depiction of the self-cleaning effect displayed by droplets rolling off the surface. (C) Liquid transport properties: an aqueous suspension of magnetic particles can be moved at will across the superhydrophobic coating surface using a magnet; the movement is highlighted by covering the surface with methyl orange powder, which is then removed by the moving droplet.

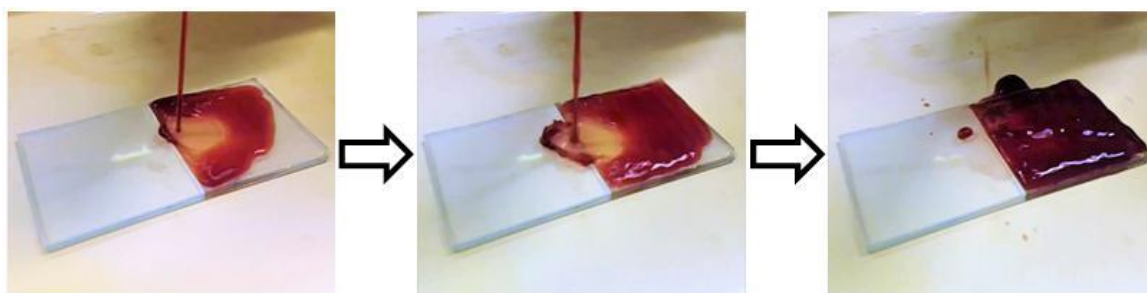


Figure 4.6 - Comparison of the behaviour of functionalized (left) and pristine (right) oxide coatings during anti-stain tests with red wine.

4.2.3 Student experience

The laboratory activity took 2-3 h, depending on the laboratory expertise of the participants, and was carried out in small groups (4-5 persons). During the waiting time for the oxide functionalization, students prepared the material needed for the application tests (dye solution, magnetic suspension, graphite powder) and familiarized with the drop-casting deposition by preparing a pristine oxide coating on a glass slide. Moreover, they carried out self-cleaning and anti-stain tests on the provided reference surfaces (uncoated glass and PTFE). All the groups were able to successfully carry out the activity and reported their results with photos and videos.

4.2.4 Follow up assessment

This experimental activity was tested by a class of Master's students in Chemical Sciences and Industrial Chemistry during the course of "Physical Chemistry of Disperse Systems and Interfaces", but it is also suitable for students with very limited lab experience, such as high school students. Thanks to its immediate and tangible results and to the limited required equipment, this laboratory experience is compatible with public engagement activities. It was tested by over 40 high school students from different backgrounds during orientation courses and by 75 high school teachers during refresher training courses. Before the hands-on activity, a one-hour seminar introduced the participants (high school students and teachers) to the basic concepts of surface science and wetting modification. The laboratory experience was successfully performed by all groups (Master students, high school students and teachers) and it was rated very high in terms of participant appreciation: 35 participants to the wetting seminar and hands-on activity ranked it the most appreciated among the 4-day refresher training course. For its simplicity, commonly available instrumentation, engaging and compelling results, this experience can be easily adapted to a wide range of teaching courses and outreach events, including science festivals.

Participants satisfaction was gauged differently depending on the group. Master's students were required to write a report concerning the laboratory activity in order to evaluate their understanding of its theoretical basis. The final course exam also included a question related to the laboratory experience and the students were later asked to complete a brief multiple-choice test. The average score was 3.5/5, while the self-evaluation regarding the understanding of theory and applications registered an average score of 4.3 and 4.4 respectively. Overall, students showed a good understanding of wetting phenomena, which profited from directly experiencing the preparation and applications of superhydrophobic surfaces and comparing their properties with conventional hydrophobic and hydrophilic materials.

A survey of the high school teachers' impressions showed a high degree of appreciation for the present hands-on activity. In the survey, some of the teachers noted that they lacked rotary evaporators to carry out the activity in their schools' laboratories. A few of the high school teachers later requested assistance to repeat the activity to their students and some of them subscribed their classes when the activity was repeated as orientation course for high-school students.

4.3 Conclusions

This hands-on activity serves the purpose of introducing students of various levels and backgrounds to the concept of wetting, one of the first steps towards the study of surface chemistry. This compact and easily reproducible experience shows the properties and potential applications of superhydrophobic materials. The surface functionalization of oxide particles with alkyl silanes was exploited to make hybrid coatings displaying Cassie-Baxter behavior via a simple and quick deposition method. The superhydrophobic coatings, along with reference hydrophobic, hydrophilic and superhydrophilic surfaces, were tested to investigate their anti-stain, self-cleaning and liquid-transport properties. Participants greatly appreciated the engaging and tangible results and the possibility of documenting their experience by taking pictures and videos with their smartphones. The application-oriented character of the activity, along with the use of every-day materials, makes it suitable for teaching laboratories as well as for public engagement activities.

4.4 References

- [1] X. Chen, S.S. Mao, Titanium Dioxide Nanomaterials: Synthesis, Properties, Modifications, and Applications, *Chem. Rev.* 107 (2007) 2891–2959. doi:10.1021/cr0500535.
- [2] L. Rimoldi, D. Meroni, E. Falletta, A.M. Ferretti, A. Gervasini, G. Cappelletti, S. Ardizzone, The role played by different TiO₂ features on the photocatalytic degradation of paracetamol, *Appl. Surf. Sci.* 424 (2017) 198–205. doi:10.1016/j.apsusc.2017.03.033.
- [3] K. Liu, M. Cao, A. Fujishima, L. Jiang, Bio-Inspired Titanium Dioxide Materials with Special Wettability and Their Applications, *Chem. Rev.* 114 (2014) 10044–10094. doi:10.1021/cr4006796.
- [4] J.-B. Chemin, S. Bulou, K. Baba, C. Fontaine, T. Sindzingre, N.D. Boscher, P. Choquet, Transparent anti-fogging and self-cleaning TiO₂/SiO₂ thin films on polymer substrates using atmospheric plasma, *Sci. Rep.* 8 (2018) 9603. doi:10.1038/s41598-018-27526-7.
- [5] A. Shakeri, D. Yip, M. Badv, S. Imani, M. Sanjari, T. Didar, Self-Cleaning Ceramic Tiles Produced via Stable Coating of TiO₂ Nanoparticles, *Materials (Basel)*. 11 (2018) 1003. doi:10.3390/ma11061003.
- [6] B.K. Tudu, A. Sinhamahapatra, A. Kumar, Surface Modification of Cotton Fabric Using TiO₂ Nanoparticles for Self-Cleaning, Oil–Water Separation, Antistain, Anti-Water Absorption, and Antibacterial Properties, *ACS Omega*. 5 (2020) 7850–7860. doi:10.1021/acsomega.9b04067.

- [7] J. Drelich, A. Marmur, Physics and applications of superhydrophobic and superhydrophilic surfaces and coatings, *Surf. Innov.* 2 (2014) 211–227. doi:10.1680/si.13.00017.
- [8] J. Huang, Y. Lai, L. Wang, S. Li, M. Ge, K. Zhang, H. Fuchs, L. Chi, Controllable wettability and adhesion on bioinspired multifunctional TiO₂ nanostructure surfaces for liquid manipulation, *J. Mater. Chem. A* 2 (2014) 18531–18538. doi:10.1039/C4TA04090B.
- [9] B.I. Stefanov, D. Lebrun, A. Mattsson, C.G. Granqvist, L. Österlund, Demonstrating Online Monitoring of Air Pollutant Photodegradation in a 3D Printed Gas-Phase Photocatalysis Reactor, *J. Chem. Educ.* 92 (2015) 678–682. doi:10.1021/ed500604e.
- [10] M.W. Pitcher, S.M. Emin, M. Valant, A Simple Demonstration of Photocatalysis Using Sunlight, *J. Chem. Educ.* 89 (2012) 1439–1441. doi:10.1021/ed300048z.
- [11] S. Gravelle, B. Langham, Photocatalysis , A Laboratory Experiment for an Integrated Physical Chemistry – Instrumental Analysis Course, *J. Chem. Educ.* 80 (2003) 911–913.
- [12] K.D. Giglio, D.B. Green, B. Hutchinson, Photocatalytic Destruction of an Organic Dye Using TiO₂ and Solar Energy: A General Chemistry Experiment, *J. Chem. Educ.* 72 (1995) 352. doi:10.1021/ed072p352.
- [13] S.P. Flynn, M. McKenna, R. Monaghan, S.M. Kelleher, S. Daniels, A. MacCormac, Aqua-Art: A Demonstration of Hydrophilic and Hydrophobic Surfaces Fabricated by Plasma Enhanced Chemical Vapor Deposition, *J. Chem. Educ.* 94 (2017) 221–225. doi:10.1021/acs.jchemed.6b00474.
- [14] J.X.H. Wong, H.-Z. Yu, Preparation of Transparent Superhydrophobic Glass Slides: Demonstration of Surface Chemistry Characteristics, *J. Chem. Educ.* 90 (2013) 1203–1206. doi:10.1021/ed300809m.
- [15] C.L. Leverette, C. Wills, M.A. Perkins, S.A. Jacobs, Structural Analysis of Nanofilms Using FTIR Spectroscopy. An Introduction to the Spectroscopic Analysis of Nanostructures for Undergraduate Students, *J. Chem. Educ.* 86 (2009) 719. doi:10.1021/ed086p719.
- [16] P. Kim, J. Alvarenga, J. Aizenberg, R.S. Sleeper, Hydroglyphics: Demonstration of Selective Wetting on Hydrophilic and Hydrophobic Surfaces, *J. Chem. Educ.* 90 (2013) 625–628. doi:10.1021/ed3003308.
- [17] W. Barthlott, M. Mail, C. Neinhuis, Superhydrophobic hierarchically structured surfaces in biology: evolution, structural principles and biomimetic applications, *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* 374 (2016) 20160191. doi:10.1098/rsta.2016.0191.
- [18] K. Liu, X. Yao, L. Jiang, Recent developments in bio-inspired special wettability, *Chem. Soc. Rev.* 39 (2010) 3240. doi:10.1039/b917112f.

- [19] G.D. Bixler, B. Bhushan, Fluid drag reduction and efficient self-cleaning with rice leaf and butterfly wing bioinspired surfaces, *Nanoscale*. 5 (2013) 7685. doi:10.1039/c3nr01710a.
- [20] X. Deng, L. Mammen, H.-J. Butt, D. Vollmer, Candle Soot as a Template for a Transparent Robust Superamphiphobic Coating, *Science* (80-.). 335 (2012) 67–70. doi:10.1126/science.1207115.
- [21] T.-S. Wong, S.H. Kang, S.K.Y. Tang, E.J. Smythe, B.D. Hatton, A. Grinthal, J. Aizenberg, Bioinspired self-repairing slippery surfaces with pressure-stable omniphobicity, *Nature*. 477 (2011) 443–447. doi:10.1038/nature10447.
- [22] K.-C. Park, P. Kim, A. Grinthal, N. He, D. Fox, J.C. Weaver, J. Aizenberg, Condensation on slippery asymmetric bumps, *Nature*. 531 (2016) 78–82. doi:10.1038/nature16956.
- [23] M. Nosonovsky, Multiscale Roughness and Stability of Superhydrophobic Biomimetic Interfaces, *Langmuir*. 23 (2007) 3157–3161. doi:10.1021/la062301d.
- [24] R. Hensel, C. Neinhuis, C. Werner, The springtail cuticle as a blueprint for omniphobic surfaces, *Chem. Soc. Rev.* 45 (2016) 323–341. doi:10.1039/C5CS00438A.
- [25] S. Wang, L. Jiang, Definition of Superhydrophobic States, *Adv. Mater.* 19 (2007) 3423–3424. doi:10.1002/adma.200700934.
- [26] J. Gao, Y. Liu, H. Xu, Z. Wang, X. Zhang, Mimicking Biological Structured Surfaces by Phase-Separation Micromolding, *Langmuir*. 25 (2009) 4365–4369. doi:10.1021/la9008027.
- [27] X. Feng, J. Zhai, L. Jiang, The Fabrication and Switchable Superhydrophobicity of TiO₂ Nanorod Films, *Angew. Chemie Int. Ed.* 44 (2005) 5115–5118. doi:10.1002/anie.200501337.
- [28] Q.F. Xu, Y. Liu, F.-J. Lin, B. Mondal, A.M. Lyons, Superhydrophobic TiO₂ – Polymer Nanocomposite Surface with UV-Induced Reversible Wettability and Self-Cleaning Properties, *ACS Appl. Mater. Interfaces*. 5 (2013) 8915–8924. doi:10.1021/am401668y.
- [29] A. Tuteja, W. Choi, M. Ma, J.M. Mabry, S.A. Mazzella, G.C. Rutledge, G.H. McKinley, R.E. Cohen, Designing Superoleophobic Surfaces, *Science* (80-.). 318 (2007) 1618–1622. doi:10.1126/science.1148326.
- [30] L. Cao, H.-H. Hu, D. Gao, Design and Fabrication of Micro-textures for Inducing a Superhydrophobic Behavior on Hydrophilic Materials, *Langmuir*. 23 (2007) 4310–4314. doi:10.1021/la063572r.
- [31] M. Nosonovsky, B. Bhushan, Superhydrophobic surfaces and emerging applications: Non-adhesion, energy, green engineering, *Curr. Opin. Colloid Interface Sci.* 14 (2009) 270–280. doi:10.1016/j.cocis.2009.05.004.
- [32] T.B. Longenberger, K.M. Ryan, W.Y. Bender, A. Krumpfer, J.W. Krumpfer, The Art of Silicones: Bringing Siloxane Chemistry to the Undergraduate Curriculum, *J. Chem. Educ.* 94 (2017) 1682–1690. doi:10.1021/acs.jchemed.6b00769.

- [33] Y.-C. Chiu, M.A. Jenks, M. Richards-Babb, B.B. Ratcliff, J.A. Juvik, K.-M. Ku, Demonstrating the Effect of Surfactant on Water Retention of Waxy Leaf Surfaces, *J. Chem. Educ.* 94 (2017) 230–234. doi:10.1021/acs.jchemed.6b00546.
- [34] V.S.J. Craig, A.C. Jones, T.J. Senden, Contact Angles of Aqueous Solutions on Copper Surfaces Bearing Self-Assembled Monolayers, 78 (2001) 345–346. doi:10.1021/ed078p345.
- [35] P. Kim, J. Alvarenga, J. Aizenberg, R.S. Sleeper, Hydroglyphics: Demonstration of Selective Wetting on Hydrophilic and Hydrophobic Surfaces, *J. Chem. Educ.* 90 (2013) 625–628. doi:10.1021/ed3003308.
- [36] C.J. Chan, K. Salaita, The Molecular Boat: A Hands-On Experiment To Demonstrate the Forces Applied to Self-Assembled Monolayers at Interfaces, *J. Chem. Educ.* 89 (2012) 1547–1550. doi:10.1021/ed200832h.
- [37] S. Verbanic, O. Brady, A. Sanda, C. Gustafson, Z.J. Donhauser, A Novel General Chemistry Laboratory: Creation of Biomimetic Superhydrophobic Surfaces through Replica Molding, *J. Chem. Educ.* 91 (2014) 1477–1480. doi:10.1021/ed4007056.
- [38] L. Rimoldi, T. Taroni, D. Meroni, Wetting Modification by Photocatalysis: A Hands-on Activity To Demonstrate Photoactivated Reactions at Semiconductor Surfaces, *J. Chem. Educ.* (2018) acs.jchemed.8b00362. doi:10.1021/acs.jchemed.8b00362.
- [39] III. An essay on the cohesion of fluids, *Philos. Trans. R. Soc. London.* 95 (1805) 65–87. doi:10.1098/rstl.1805.0005.
- [40] R.N. Wenzel, RESISTANCE OF SOLID SURFACES TO WETTING BY WATER, *Ind. Eng. Chem.* 28 (1936) 988–994. doi:10.1021/ie50320a024.
- [41] A.B.D. Cassie, S. Baxter, Wettability of porous surfaces, *Trans. Faraday Soc.* 40 (1944) 546. doi:10.1039/tf9444000546.
- [42] D. Quéré, Wetting and Roughness, *Annu. Rev. Mater. Res.* 38 (2008) 71–99. doi:10.1146/annurev.matsci.38.060407.132434.
- [43] D. Quéré, Non-sticking drops, *Reports Prog. Phys.* 68 (2005) 2495–2532. doi:10.1088/0034-4885/68/11/R01.
- [44] L. Gao, T.J. McCarthy, How Wenzel and Cassie Were Wrong, *Langmuir.* 23 (2007) 3762–3765. doi:10.1021/la062634a.
- [45] F. Milanese, G. Cappelletti, R. Annunziata, C.L. Bianchi, D. Meroni, S. Ardizzone, Siloxane-TiO₂ hybrid nanocomposites. the structure of the hydrophobic layer, *J. Phys. Chem. C.* 114 (2010) 8287–8293. doi:10.1021/jp1014669.
- [46] D. Meroni, S. Ardizzone, G. Cappelletti, M. Ceotto, M. Ratti, R. Annunziata, M. Benaglia, L. Raimondi, Interplay between Chemistry and Texture in Hydrophobic TiO₂ Hybrids, *J. Phys. Chem. C.* 115 (2011) 18649–18658. doi:10.1021/jp205142b.

[47] G. Soliveri, R. Annunziata, S. Ardizzone, G. Cappelletti, D. Meroni, Multiscale rough titania films with patterned hydrophobic/oleophobic features, *J. Phys. Chem. C*. 116 (2012) 26405–26413. doi:10.1021/jp309397c.

CHAPTER 5

Wetting modification by photocatalysis: a hands-on activity to demonstrate photoactivated reactions at semiconductor surfaces

In line with the scope of the previous Chapter, a hands-on activity focusing on the modification of the TiO_2 surface properties by photocatalysis is here reported. On one hand, the effect of light irradiation on the wetting properties of pristine TiO_2 surfaces is investigated for antifogging applications. On the other hand, the photocatalyst surface properties are modified by grafting with alkylsilanes and then patterned films with wetting contrast are prepared by photocatalytic lithography to obtain the site-selective adsorption of dye molecules.

This activity was tested by students of the first year of the Master's degrees in Chemical Sciences and Industrial Chemistry during a teaching laboratory of the course 'Physical Chemistry of Disperse Systems and Interfaces', at mid-semester after an introductory lecture. Nonetheless, thanks to the procedure simplicity, inexpensive instrumentation and tangible results, this experience was easily adapted for high-school students and refresher courses for high school teachers. It is also well suited for demonstrations at science festivals.

EXPERIMENTAL OVERVIEW

TiO_2 surface is hydrophilic since it exposes polar moieties such as hydroxyl groups, which can form strong interactions with water. As previously mentioned, the hydrophilicity of TiO_2 surfaces can be enhanced by UV irradiation, leading to complete water spreading (*superhydrophilicity*).

Surface hydroxylation of TiO_2 can be exploited to graft alkylsilane derivatives via the formation of Si–O–Ti bonds (Figure 5.1), conferring hydrophobicity to the oxide surface[14]. By this approach, water contact angles higher than 150° (*superhydrophobicity*) can be achieved when films made of TiO_2 micro/nanoparticles are functionalized with long-chain or perfluorinated alkylsilanes. The resulting superhydrophobic behavior is due to the combination of inherently hydrophobic alkyl/perfluorinated chains and of the surface roughness imparted by the TiO_2 micro/nanoparticles, according to the Cassie-Baxter model[15].

Photocatalysis offers a tool to prepare patterned surfaces with superhydrophobic/superhydrophilic contrast[16–18]. By irradiating through a photomask a TiO_2 film functionalized with alkylsilanes (Figure 5.1), the alkyl chains

can be degraded in the light-exposed areas, leading to superhydrophilic patches[19]. The film parts covered by the photomask are instead unaffected, retaining the superhydrophobic behavior. The resulting wetting contrast can then be revealed by pouring an aqueous solution onto the patterned surface.

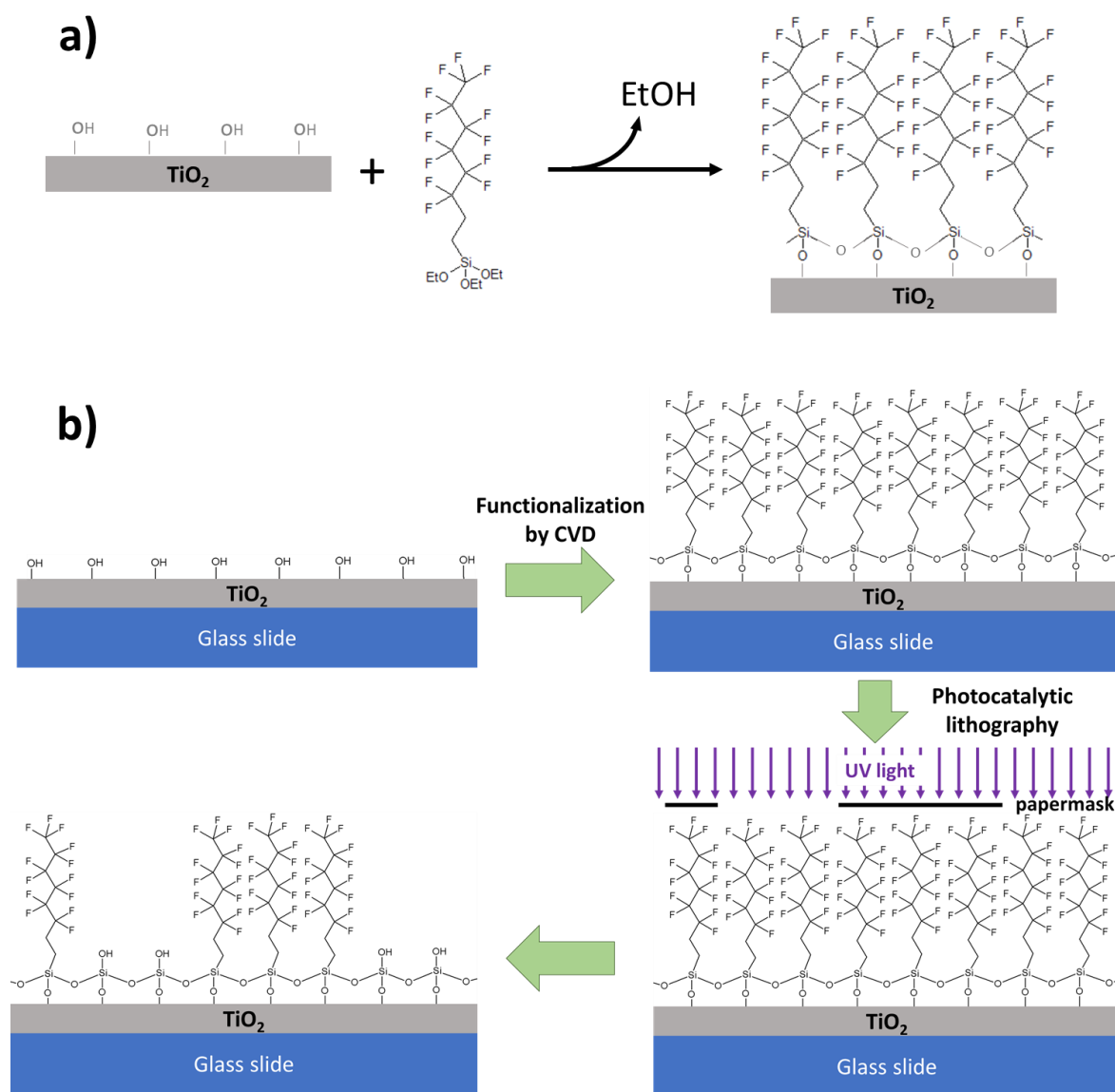


Figure 5.1 – Schematic of the TiO_2 film functionalization (A) and photocatalytic lithography (B).

5.1 Experimental procedures

5.1.1 Materials and Instrumentation

Commercial titanium dioxide (Aeroxide® TiO₂ P25) was purchased from Evonik. Acetone, 2-propanol, 1*H*,1*H*,2*H*,2*H*-perfluorooctyl-triethoxysilane (PFOS) and bromophenol blue were acquired from Sigma-Aldrich. Doubly distilled MilliQ water was used to prepare the dye solutions. The chemical vapor deposition (CVD) requires a 20 cm Pyrex glass Petri dish and an oven. Lithographic experiments were optimized using a 500 W iron halogenide lamp (Jelosil HG500); although they were not tested in this instance, less powerful UV lamps (*e.g.*, UV torches or TLC lamps)[20] might be employed by increasing the irradiation time. Film deposition was carried out on soda lime glass slides, using a spin coater (SPS-Europe). Cleaning of the supports and suspension preparation were carried out using an ultrasonic bath. Contact angle measurements were performed on a Krüss EasyDrop instrument.

5.1.2 Preparation of TiO₂ films

Five glass slides (5 x 5 cm²) were cleaned by sequentially sonicating in acetone, 2-propanol and water, then dried on a hot plate. A TiO₂ suspension in 2-propanol (0.5 g in 10 mL) was prepared by sonication. TiO₂ films were deposited by dispensing 2 mL of TiO₂ suspension drop-wise on the glass slides, then spin coated adopting the following parameters: rotation time 20 s, rotation rate 3000 rpm and rotation acceleration/deceleration 500 rpm/s. After the deposition, films were dried in an oven at 80°C for 5 min to remove any residual solvent. Alternatively, the TiO₂ films can be deposited by drop casting using a less concentrated TiO₂ slurry, although in that case a higher PFOS amount might be necessary for the functionalization.

5.1.3 Functionalization of TiO₂ films with perfluoroalkylsilane

The functionalization of TiO₂ films was carried out by CVD (Figure 5.2A), according to a previously reported procedure[21]. Two of the TiO₂ films were placed on a Petri dish base together with a Teflon cup containing 250 µL of pure PFOS (Figure 5.2B). The Petri dish was then covered with its lid to create a closed chamber (Figure 5.2C), which was placed in an oven at 100°C for 2 h. The films were then removed from the oven and allowed to cool at room temperature before measuring their water contact angle.

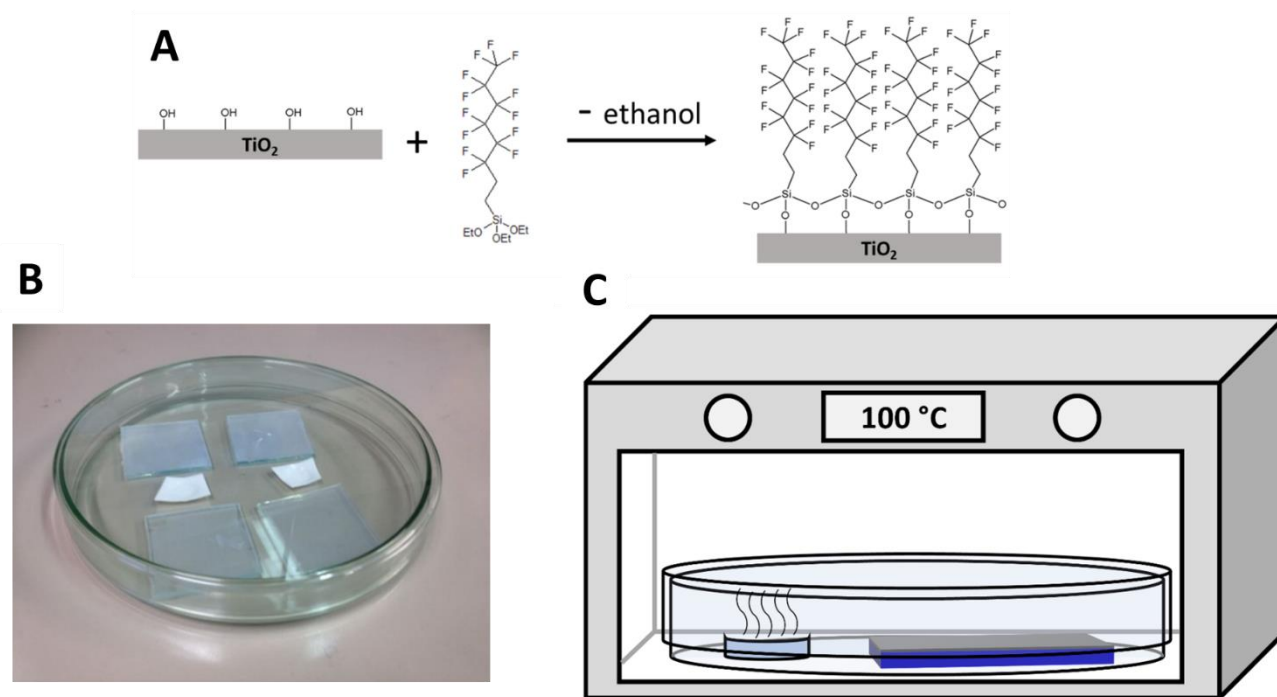


Figure 5.2 – Reaction of TiO_2 films with PFOS (A); CVD experimental set up (B, C).

5.1.4 Testing of anti-fogging properties

One of the bare TiO_2 films was irradiated for 30 min under UV light, while the other was kept in the dark. The water contact angle of the two films was measured (using 5–10 μL droplets) and compared with that of the glass slides. Then, the antifogging properties of the irradiated and non-irradiated films were tested. The film fogging was promoted by placing the TiO_2 layers over a beaker filled with hot water.

5.1.5 Photocatalytic lithography

One of the functionalized TiO_2 films was covered with a photomask, prepared by cutting the desired pattern in a paper sheet. Then, the covered film was irradiated under UV light for 45 min. At the end of the irradiation step, the wetting contrast between the mask-covered areas (remained superhydrophobic) and the exposed regions (become superhydrophilic) was highlighted by depositing a dye aqueous solution (10 mg in 100 mL).

5.2 Results and discussion

5.2.1 Antifogging properties

Water contact angles were measured using a digital contact angle goniometer equipped with a software-controlled microsyringe. In case an instrument for contact angle measurements is not available, students can dispense the water droplets using micropipettes, then they can acquire the droplets images using a smartphone or a camera

with a magnifying lens and determine the water contact angle between the surface and the tangent to the droplet profile, using a free goniometer software or app, such as BeforeOffice Snap.

Water contact angles of glass slides were $18^\circ \pm 2^\circ$, while those on pristine TiO_2 films before and after irradiation were $12^\circ \pm 2^\circ$ (Figure 5.3) and $<5^\circ$ (complete spreading), respectively (Figure 5.4, insets). Antifogging properties mirrored the observed differences in water contact angle. The bare glass surface got immediately covered in droplets upon steam exposure (Figure 5.4A), impairing the glass transparency. In the case of non-irradiated TiO_2 films, fogging effects were still appreciable. Care must be taken to avoid accidental exposure of the TiO_2 films to direct sunlight or powerful lamps before the experiment to avoid inducing superhydrophilicity also in the non-UV irradiated film. After irradiation under UV lamp, TiO_2 films showed instead excellent antifogging behavior (Figure 5.4B): irradiated films retained their visual clarity upon steam exposure, as condensed water droplets immediately spread onto the TiO_2 surface creating a continuous water film. It should be noted that irradiation of the glass slide with a UV lamp does not alter its wetting features as SiO_2 is an insulator.

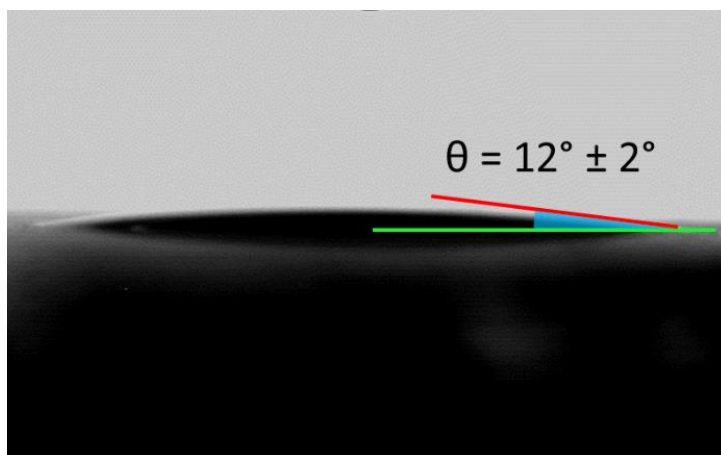


Figure 5.3 – Water contact angle measurement of a TiO_2 film before UV irradiation.

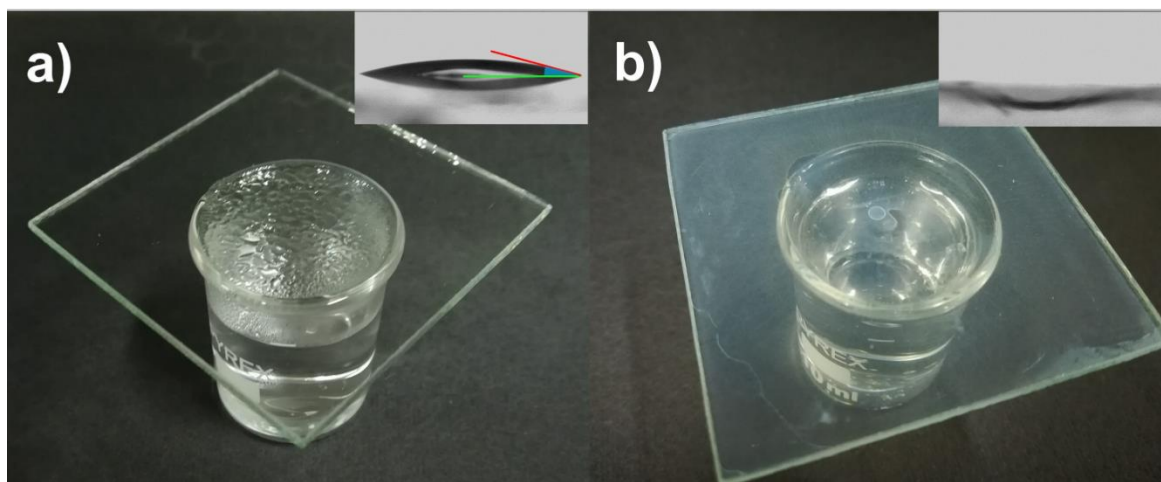


Figure 5.4 – (A) Fogging of a glass slide placed over a hot water beaker. (B) Antifogging properties of a UV irradiated TiO_2 film. Insets: corresponding water contact angles; in the case of the irradiated TiO_2 film, no droplet profile can be appreciated due to the complete spreading of water.

5.2.2 Surface functionalization and photocatalytic lithography

TiO₂ films were functionalized by CVD using a perfluorinated alkylsilane, PFOS. The grafting mechanism involves the formation of silanol moieties from the reaction of PFOS with trace amount of water adsorbed onto the film surface; then silanols undergo condensation reaction with surface hydroxyls and with neighboring silanol molecules, giving rise to a grafted organic layer that imparts hydrophobic properties to the TiO₂ film. The average observed contact angle was $160^{\circ} \pm 2^{\circ}$, characteristic of superhydrophobic materials and indicative of the formation of a Cassie-Baxter state. The measurement of such high contact angles can be complex, as water droplets tend to roll away before any measurements can be performed. In these cases, instead of dispensing a water droplet onto the solid surface using the syringe, the substrate should be lifted till it contacts the water droplet hanging from the syringe tip.

PFOS can be replaced by the less expensive non-fluorinated octyltriethoxysilane, although in this case the resulting contact angles might be lower[21].

The functionalized superhydrophobic films were patterned using the photocatalytic lithography technique. Several materials can be used for the photomask preparation (e.g., paper, metals, ...) if they are effective in shielding UV light. Students really enjoyed the creation of photomask from their own design (Figure 5.5). The wetting contrast can be revealed dripping water or a dye aqueous solution onto the film.

The lateral resolution of the technique can be investigated by using photomask grids with controlled spacing and revealing the wetting contrast using the steam condensation on the film surface and an optical microscope.

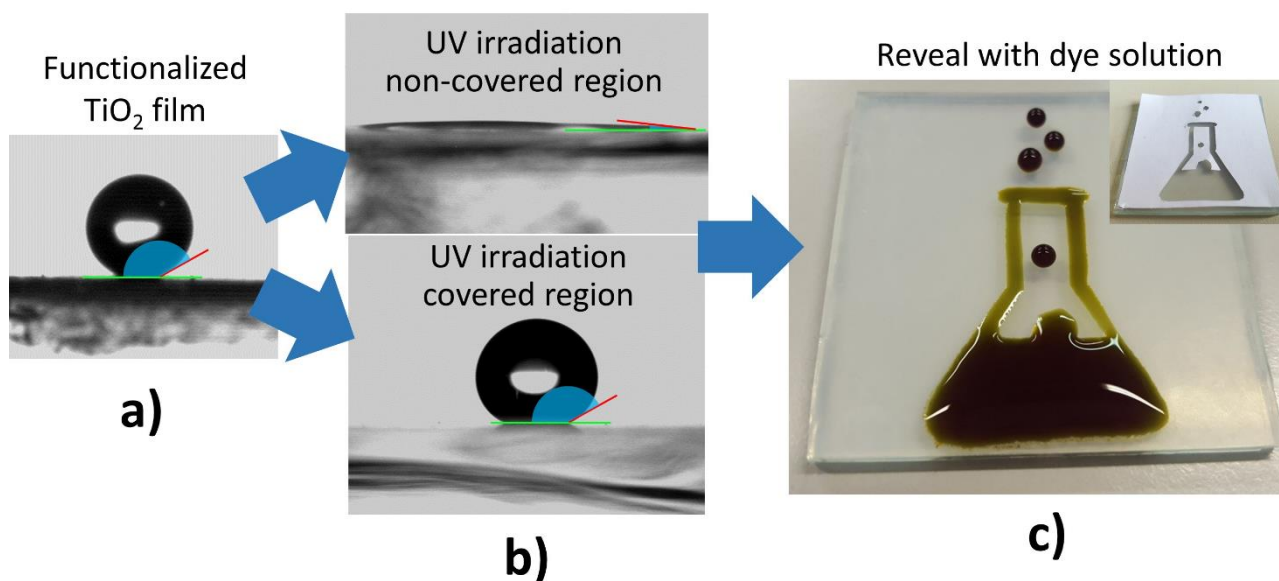


Figure 5.5 – Water contact angles of the functionalized TiO₂ film before irradiation (A); difference between covered and non-covered regions of the film after irradiation using a photomask (B); a picture of the resulting patterned surface after revealing with a bromophenol blue solution (C) (inset: photomask design).

5.2.3 Student experience

The activity was performed in small groups and required 4 hours. The participants first deposited the TiO₂ films and functionalized two of them via CVD. During the time required by the CVD, students became familiar with contact angle measurements, performed antifogging tests on the pristine TiO₂ films, prepared the dye solution and designed their own photomasks. The activity showed excellent reproducibility among all the students' groups, who documented their results using photos and videos later implemented in a laboratory report.

5.2.4 Follow up assessment

At the end of the laboratory activity, students were asked to write a report, aimed at evaluating their learning level. Moreover, since the experience was part of a Master's degree course, the final exam included some questions pertaining the laboratory experience. Additionally, a survey was held to gather students' impressions: the overall reception was positive, scoring an average of 8.6 /10, and all students were keen on recommending this activity to others. Generally, students were satisfied with the provided theoretical explanations and the overall organization. In particular, some of them underlined that time schedules were respected and no hurdles were encountered; this may suggest the experience can be easily adapted for undergraduates as well. However, few students found the functionalization time too long: the dead times could be put to use by further explaining to the students the theory and applications of photocatalysis and superhydrophilic/superhydrophobic materials.

5.3 Conclusions

This hands-on activity displays photocatalysis potential to modify the wetting properties of TiO₂ films. Functionalization with alkylsilanes and UV irradiation were exploited to obtain superhydrophobic and superhydrophilic TiO₂-based films, respectively. The combination of the two treatments was also adopted to create patterned surfaces via photocatalytic lithography. The aim of the experience was to introduce students to photocatalysis basics and give them a grasp on surface science, mainly related to wetting phenomena. An engaging atmosphere was achieved by relating theoretical aspects and experimental work to everyday applications, thus improving the learning process, as demonstrated by the students' positive response.

5.4 References

- [1] X. Chen, S.S. Mao, Titanium Dioxide Nanomaterials: Synthesis, Properties, Modifications, and Applications, *Chem. Rev.* 107 (2007) 2891–2959. doi:10.1021/cr0500535.
- [2] L. Rimoldi, D. Meroni, E. Falletta, A.M. Ferretti, A. Gervasini, G. Cappelletti, S. Ardizzone, The role played by different TiO₂ features on the photocatalytic

degradation of paracetamol, *Appl. Surf. Sci.* 424 (2017) 198–205. doi:10.1016/j.apsusc.2017.03.033.

[3] K. Liu, M. Cao, A. Fujishima, L. Jiang, Bio-Inspired Titanium Dioxide Materials with Special Wettability and Their Applications, *Chem. Rev.* 114 (2014) 10044–10094. doi:10.1021/cr4006796.

[4] J. Drelich, A. Marmur, Physics and applications of superhydrophobic and superhydrophilic surfaces and coatings, *Surf. Innov.* 2 (2014) 211–227. doi:10.1680/si.13.00017.

[5] K. Nakata, A. Fujishima, TiO₂ photocatalysis: Design and applications, *J. Photochem. Photobiol. C Photochem. Rev.* 13 (2012) 169–189.

[6] B.I. Stefanov, D. Lebrun, A. Mattsson, C.G. Granqvist, L. Österlund, Demonstrating Online Monitoring of Air Pollutant Photodegradation in a 3D Printed Gas-Phase Photocatalysis Reactor, *J. Chem. Educ.* 92 (2015) 678–682. doi:10.1021/ed500604e.

[7] M.W. Pitcher, S.M. Emin, M. Valant, A Simple Demonstration of Photocatalysis Using Sunlight, *J. Chem. Educ.* 89 (2012) 1439–1441. doi:10.1021/ed300048z.

[8] S. Gravelle, B. Langham, Photocatalysis , A Laboratory Experiment for an Integrated Physical Chemistry – Instrumental Analysis Course, *J. Chem. Educ.* 80 (2003) 911–913.

[9] K.D. Giglio, D.B. Green, B. Hutchinson, Photocatalytic Destruction of an Organic Dye Using TiO₂ and Solar Energy: A General Chemistry Experiment, *J. Chem. Educ.* 72 (1995) 352. doi:10.1021/ed072p352.

[10] S.P. Flynn, M. McKenna, R. Monaghan, S.M. Kelleher, S. Daniels, A. MacCormac, Aqua-Art: A Demonstration of Hydrophilic and Hydrophobic Surfaces Fabricated by Plasma Enhanced Chemical Vapor Deposition, *J. Chem. Educ.* 94 (2017) 221–225. doi:10.1021/acs.jchemed.6b00474.

[11] J.X.H. Wong, H.-Z. Yu, Preparation of Transparent Superhydrophobic Glass Slides: Demonstration of Surface Chemistry Characteristics, *J. Chem. Educ.* 90 (2013) 1203–1206. doi:10.1021/ed300809m.

[12] C.L. Leverette, C. Wills, M.A. Perkins, S.A. Jacobs, Structural Analysis of Nanofilms Using FTIR Spectroscopy. An Introduction to the Spectroscopic Analysis of Nanostructures for Undergraduate Students, *J. Chem. Educ.* 86 (2009) 719. doi:10.1021/ed086p719.

[13] P. Kim, J. Alvarenga, J. Aizenberg, R.S. Sleeper, Hydroglyphics: Demonstration of Selective Wetting on Hydrophilic and Hydrophobic Surfaces, *J. Chem. Educ.* 90 (2013) 625–628. doi:10.1021/ed3003308.

[14] D. Meroni, L. Lo Presti, G. Di Liberto, M. Ceotto, R.G. Acres, K.C. Prince, R. Bellani, G. Soliveri, S. Ardizzone, A Close Look at the Structure of the TiO₂-APTES Interface in Hybrid Nanomaterials and Its Degradation Pathway: An Experimental and

Theoretical Study, J. Phys. Chem. C. 121 (2017) 430–440. doi:10.1021/acs.jpcc.6b10720.

[15] A.B.D. Cassie, S. Baxter, Wettability of porous surfaces, Trans. Faraday Soc. 40 (1944) 546. doi:10.1039/tf9444000546.

[16] A. Fujishima, X. Zhang, D. Tryk, TiO₂ photocatalysis and related surface phenomena, Surf. Sci. Rep. 63 (2008) 515–582. doi:10.1016/j.surfrep.2008.10.001.

[17] Y. Paz, Self-assembled monolayers and titanium dioxide: From surface patterning to potential applications., Beilstein J. Nanotechnol. 2 (2011) 845–61. doi:10.3762/bjnano.2.94.

[18] Y. Paz, Preferential photodegradation – why and how?, Comptes Rendus Chim. 9 (2006) 774–787. doi:10.1016/j.crci.2005.03.032.

[19] G. Soliveri, R. Annunziata, S. Ardizzzone, G. Cappelletti, D. Meroni, Multiscale rough titania films with patterned hydrophobic/oleophobic features, J. Phys. Chem. C. 116 (2012) 26405–26413. doi:10.1021/jp309397c.

[20] V. Pifferi, G. Soliveri, G. Panzarasa, S. Ardizzzone, G. Cappelletti, D. Meroni, L. Falciola, Electrochemical sensors cleaned by light: a proof of concept for on site applications towards integrated monitoring systems, RSC Adv. 5 (2015) 71210–71214. doi:10.1039/C5RA12219H.

[21] G. Soliveri, V. Pifferi, R. Annunziata, L. Rimoldi, V. Aina, G. Cerrato, L. Falciola, G. Cappelletti, D. Meroni, Alkylsilane-SiO₂ Hybrids. A Concerted Picture of Temperature Effects in Vapor Phase Functionalization, J. Phys. Chem. C. 119 (2015) 15390–15400. doi:10.1021/acs.jpcc.5b04048.

CHAPTER 6

Synthesis of porous TiO₂ films as photo-regenerating anti-fouling coatings for electrochemical sensors

After briefly exploring the world of TiO₂ surface modification with a didactic approach, I devoted great efforts to developing TiO₂ films as functional coatings. As mentioned earlier, when designing active surfaces their surface extension plays a role of utmost importance, as for several applications the interface must be as wide as possible to maximise interfacial phenomena. With this consideration in mind, I started working on the preparation of porous TiO₂ films for their application as functional coatings.

Titania-based porous coatings have garnered significant attention in the last few years because of their remarkable optical and electrochemical properties, such as a high refractive index, dielectric constant, permittivity and excellent transmittance of visible light[1,2]. These features, together with the larger surface area provided by their porous structure, have driven the research on these films towards a broad range of applications: from high efficiency photoelectrodes in dye sensitized solar cells[3], to support matrixes for surface-assisted laser desorption/ionization mass spectroscopy[4], to electrochemical capacitors for energy storage[5] and gas sensors for detecting and monitoring toxic chemicals[6].

Interestingly, only few research works reported the use of TiO₂ films to develop photo-renewable coatings to protect sensors[7–9], even though, in recent years, increasing attention has been devoted to the design of smart sensing surfaces with antifouling properties[10,11]. Indeed, electrode fouling is considered one of the main issues in the sensors field, especially in the analysis of biological matrices, due to the inherent complexity of physiological fluids and the high concentration of interferents compared to analytes[12,13], which induces a decrease in the sensitivity and life-time of sensors. Surface fouling finally leads to sensor failure as a consequence of the interruption of analyte transport to the sensing layer[14].

In this regard, the use of porous silica-based coatings acting as interference-abating layers, to protect dopamine sensors from the fouling action of large proteins such as mucin, was recently reported[15]. While those coatings prevented the immediate fouling of the sensor surface at the hands of mucin through their size-exclusive structure, they could not get rid of it once it started accumulating on their surface.

To solve this issue, I investigated the deposition of porous TiO₂ films suited to catalyse the photo-induced degradation of organic impurities while not impairing the electrochemical activity of the sensor beneath. To this aim, films were deposited on FTO glass slides and their conductive properties were characterized by means of cyclic voltammetry, to gauge their effect on the underlying electrode surface.

To preserve the electrochemical properties of the electrode underneath, a highly porous surface is required, as not to block analytes from reaching the electrode surface. On top of that, a well-developed pore network ensures a much greater number of photocatalytic and adsorption sites compared to dense surfaces. However, control over pore size and structure is technically difficult to achieve while keeping an optimal film thickness without yielding delaminating or cracking.

Among the various techniques traditionally adopted to produce porous films, one that offers optimal control over porosity is template-assisted deposition[6,16,17]. In this work, TiO₂ porous films were prepared through multiple deposition procedures all revolving around the use of differently sized polystyrene (PS) nanospheres, both commercial and synthesised in-house by a classical procedure[18], which acted as hard templates. The adopted deposition techniques included spin-coating, both of hybrid SiO₂/TiO₂ and pure titania films, and evaporation-induced self-assembly (EISA). The effect of many synthetic parameters, such as spinning speed, precursor concentration and evaporation temperature, was investigated. Moreover, the photo-regenerative properties of all deposited films were tested by artificially fouling their surface with a long-chained organosilane[19].

6.1 Experimental procedures

6.1.1 Materials

Chemicals were acquired from Sigma-Aldrich (unless differently stated) and were used without further purification. Polystyrene (PS) latex suspensions (10% m/v) of three different diameters (30, 60 and 100 nm) were purchased from Magsphere Inc. (Pasadena, CA, USA). The PS latex beads were not functionalized, but the suspension was stabilized by anionic surfactants. Fluorine-doped tin oxide (FTO) covered glass slides ($\sim 7 \text{ } \Omega/\text{sq}$, Sigma-Aldrich, 2 x 2 cm) and borosilicate glass slides were used as substrates for film depositions. Water was doubly distilled and deionised through a Milli-Q apparatus.

6.1.2 SiO₂ sol preparation

A stable SiO₂ sol was prepared at room temperature, following a previously reported synthetic procedure[20]. 16.8 mL of Milli-Q water, 25.4 mL of ethanol, 2.2 mL of a 1 M HCl aqueous solution and 4.9 mL of tetraethyl orthosilicate were mixed at room temperature for 1 hour. The sol was then aged for 24 hours before use and stored at 5°C.

6.1.3 TiO₂ sol preparation

A stable TiO₂ sol was prepared at room temperature, adapting a previously reported synthetic procedure[21]. At first, 126.24 g of ethanol was introduced in a flask, then the following reagents were added under magnetic stirring in this order: 27.28 g of titanium(IV) isopropoxide (Ti(*i*PrO)₄), 1 mL of 37% aqueous HCl solution and a previously prepared solution of 0.47 g of Lutensol ON 70 in 102.57 g of ethanol. The reaction mixture was then stirred for one hour.

6.1.4 Synthesis of polystyrene nanospheres

The largest polystyrene nanospheres (350 and 900 nm in diameter) were prepared by Dr. V. Sabatini adapting a synthetic procedure from the literature[18], based on a free radical polymerization reaction. The growth of PS particles was controlled by tuning reaction conditions, specifically the amount of initiator, the ionic strength and the reaction temperature. In a typical synthesis, a certain amount of styrene was added to 100 mL of water in a N₂ atmosphere. Then, an aqueous solution of K₂SO₄, the initiator, was added and the mixture was stirred for 24 h at a set temperature. Table 6.1 reports the parameters adopted to prepare PS nanospheres of various sizes.

Name	Styrene (mmol L ⁻¹)	K ₂ SO ₄ (mmol L ⁻¹)	Temperature (°C)
PS1	570	0.45	95
PS2	570	0.29	95
PS3	870	2.2	60

Table 6.1 - Reaction parameters for PS suspension preparation.

6.1.5 Film deposition

Before film deposition, FTO and borosilicate glass supports were cleaned by subsequent washings with acetone, 2-propanol and Milli-Q water in an ultrasound bath. Substrates were then immersed for 30 s in concentrated sulfuric acid (98%), rinsed with Milli-Q water and dried in the oven at 150 °C.

6.1.5.1 Deposition of SiO₂/TiO₂ composite films by spin-coating

The deposition procedure for SiO₂ films had been previously reported[15]. 1 mL of SiO₂ sol and 830 µL of commercial PS latex (30, 60 or 100 nm) were mixed together. A variable number of layers (one or three) were deposited by a spin-coater apparatus (3000 rpm, 500 rpm/s, 20 s). After drying at room temperature, PS templates were removed by immersing the film in chloroform for 3 h. The film was then washed with ethanol and dried in an oven at 80 °C for 24 h.

Then, a TiO₂ layer was deposited on top of the SiO₂ ones by spin-coating a set amount of TiO₂ sol. The film was then calcined at 400°C for 4 hours in O₂ flux (9 NL h⁻¹).

Samples were named according to the size of PS templates and the number of layers, as reported in Table 6.2:

Name	Template size (nm)	SiO ₂ layers
1SP60T	60	1
3SP30T	30	3
3SP60T	60	3
3SP100T	100	3

Table 6.2 - Sample list for SiO₂/TiO₂ composite films.

6.1.5.2 Deposition of TiO₂ films by slow evaporation

This deposition procedure was adapted from the literature[17]. A suspension, containing 40 mL of Milli-Q water, 2 mL PS3 latex suspension and 300 μ L of titanium(IV) bis(ammonium lactato)dihydroxide (TiBALDH) was prepared. The glass support was immersed in the suspension, which was then placed in an oven at either 65 °C or 90 °C on a home-made support in order to dampen vibrations. After solvent evaporation, which depending on the chosen temperature took from 24 to 48 h, the film was calcined at 400°C for 4 hours under O₂ flux (9 NL h⁻¹). Sample list is reported in Table 6.3:

Name	Template size (nm)	Temperature (°C)
TiBALDHP100	100	65
TiBALDHP600	900	65
TiBALDHP600	900	90
TiBALDHP300	350	90

Table 6.3 - Sample list for evaporation-induced TiO₂ films.

6.1.5.3 Deposition of TiO₂ films by spin-coating

In order to spin-coat the TiO₂ film, a non-aqueous titania sol was used. To ensure compatibility with the non-aqueous sol, a solvent exchange was thus performed on the PS suspensions. Preliminary tests showed that settling the particles through centrifugation to change the dispersant was not feasible, as PS nanospheres aggregated irreversibly after the procedure. For this reason, a less stressful protocol was developed: 400 μ L of ethylene glycol was put in a flat-bottomed vial, marking the liquid level on the glass. Then, 2 mL of water-based polystyrene latex (PS1 or PS2), containing 3.25 mg of Lutensol ON 70, was added and the mixture was kept evaporating in a vented oven at 50°C for several hours, until the level of the liquid reached the mark on the glass. The so-prepared PS suspension was mixed with a certain amount of TiO₂ sol and ethanol. The respective amounts were varied as reported in Table 6.4.

PS latex (μL)	TiO ₂ sol (μL)	Ethanol (μL)	TiO ₂ sol final concentration (mol L^{-1})
100	300	200	0.14
100	500	200	0.17
100	700	200	0.20

Table 6.4 - Relative amounts of components used in the preparation of the deposition suspension.

The resulting suspensions were immediately deposited on FTO glass by spin-coating. Spin-coating rotational speed was varied from 1000 to 3000 rpm, with an acceleration of 500 rpm/s for 20 s. Films were then calcined at 400 °C for 4 hours under O₂ flux (9 NL h⁻¹).

Samples from this preparation route are named after the size of PS templates, which are relatively large due to availability and characterization reasons, the rpm value and the concentration of TiO₂ sol, as reported in Table 6.5:

Name	Template size (nm)	Rotational speed (rpm)	TiO ₂ sol final concentration (mol L^{-1})	Layers
TP200_1000_0.14	200	1000	0.14	1
TP200_1500_0.14	200	1500	0.14	1
TP200_2000_0.20	200	2000	0.20	1
TP200_3000_0.14	200	3000	0.14	1
TP200_1500_0.20_1L	200	1500	0.20	1
TP200_1500_0.20_2L	200	1500	0.20	2
TP200_1500_0.20_3L	200	1500	0.20	3
TP350_1500_0.17	350	1500	0.17	1
TP350_1500_0.20	350	1500	0.20	1
TP350_3000_0.17	350	3000	0.17	1
TP350_3000_0.20	350	3000	0.20	1

Table 6.5 - Sample list for spin-coated TiO₂ films.

6.1.6 Photo-regeneration tests

Photoregeneration tests were performed using a previously reported procedure[19]. Firstly, reproducible alkylsilane layers were deposited on the film surface via chemical vapor deposition using the following procedure. Films were placed inside a closed glass chamber containing a PTFE crucible filled with 100 μL of triethoxy(octyl)silane. The chamber was then placed in an oven at 100°C for two hours. At the end of the reaction, films were washed in an ultrasonic bath for five minutes with toluene, to remove excess alkylsilane. Functionalized films were then irradiated by a 500 W iron halogenide UV lamp (Jelosil HG500) set at 375 nm. The degradation of the alkyl chains was monitored via static water contact angle measurements (2 μL droplets on a Krüss EasyDrop instrument) every 10 minutes.

6.1.7 Sample characterization

Average particle size determinations were performed on PS latex samples dispersed in Milli-Q water by Dynamic Light Scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments), operating at $\lambda = 633$ nm with a solid state He-Ne laser at a scattering angle of 173°. Each hydrodynamic diameter was averaged from at least three measurements.

Transmission electron microscopy (TEM) images of the PS particles were acquired using a Zeiss LEO 912ab Energy Filtering TEM operating at an acceleration voltage of 120 kV, equipped with a CCD-BM/1K system. Samples were dispersed in either 2-propanol or water (1 mg mL⁻¹) and deposited on Cu holey carbon grids (200 mesh).

¹H NMR spectra of the PS suspensions were collected at 25 °C with a BRUKER 400 MHz spectrometer. Samples for the analyses were prepared dissolving 10–15 mg of PS latex samples in 1 cm³ of CDCl₃.

Scanning electron microscopy (SEM) images of the films were acquired using a Hitachi TM1000 SEM operating at 15 kV. Energy-dispersive X-ray spectroscopy (EDX) analyses were carried out using a Hitachi ED3000 probe equipped on the same instrument.

Electrochemical characterization was performed in a three-electrode cell, using a saturated calomel electrode, a Pt wire and the coated FTO glass as reference, counter and working electrodes, respectively. Potassium chloride 0.1 M was used as supporting electrolyte while [Ru(NH₃)₆]³⁺/[Ru(NH₃)₆]²⁺ 3 mM as redox probe.

6.2 Results and discussion

6.2.1 Characterization of synthesised polystyrene nanospheres

In order to study the effect of pore size on the properties of porous TiO₂ films, a wide range of template sizes were tested. For this reason, apart from commercial PS latexes, measuring between 30 nm and 100 nm, larger PS templates were synthesised in-house,

adapting a previously reported procedure[22]. Size distributions were evaluated both with DLS measurements, reported in Figure 6.1, and TEM image analyses, Figure 6.2: as shown in the figures, three different particles sizes were achieved, around 200 nm (PS1 sample), 350 nm (PS2) and 900 nm (PS3).

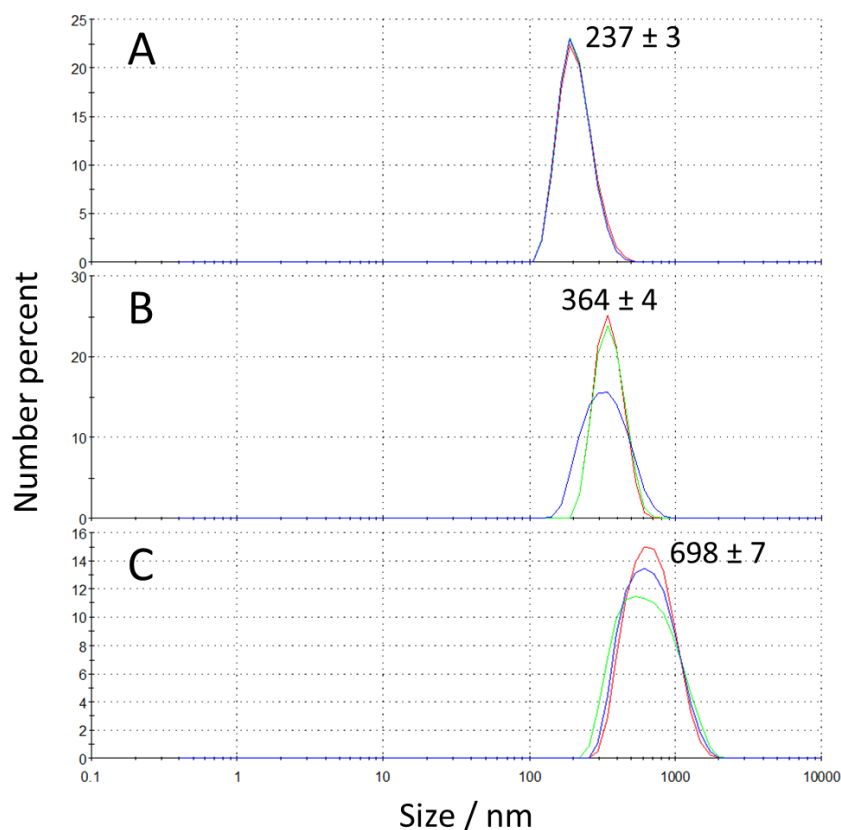


Figure 6.1 - Size distributions of synthesised PS latexes expressed as number percent: PS1 (A), PS2 (B) and PS3 (C).

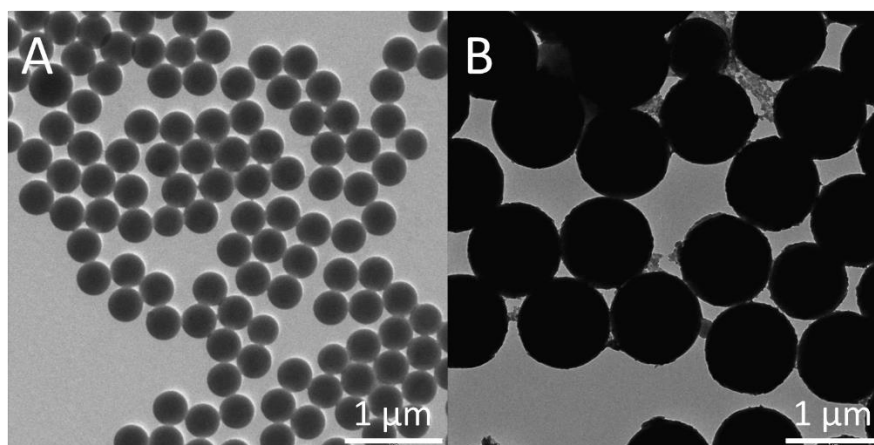


Figure 6.2 - TEM images of PS2 (A) and PS3 (B) PS latexes.

The purity of synthesised PS nanospheres was assessed through ^1H NMR analysis. As a representative example, the spectrum of PS1 latex is reported in Figure 6.3. The sample

shows a high level of purity and monomer conversion, as demonstrated by the absence of any signal belonging to residual styrene, which should fall around 5.5 ppm.

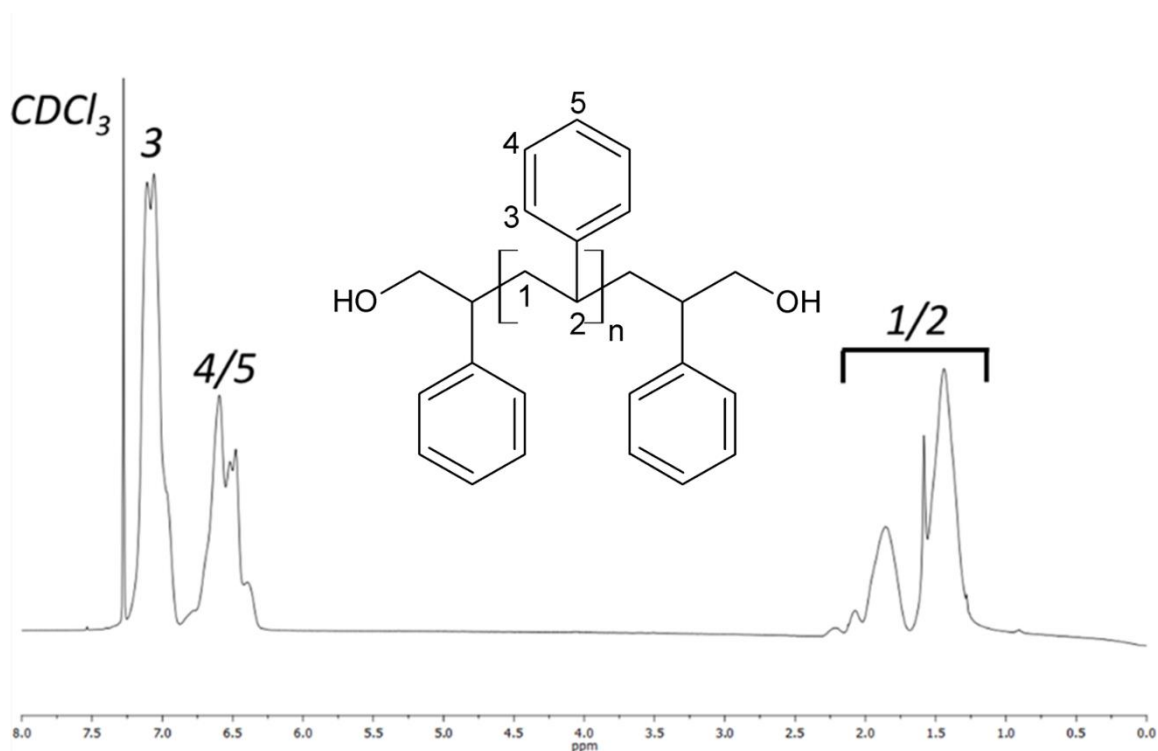


Figure 6.3 - ^1H NMR spectrum of PS1 latex.

6.2.2 Preparation and morphological characterization of TiO_2 films

6.2.2.1 $\text{SiO}_2/\text{TiO}_2$ composite films

In keeping with the previous work on SiO_2 porous coatings for electrochemical sensors[15], the first TiO_2 film deposition approach aimed at conferring photocatalytic properties to these silica-based systems. With this purpose, after depositing porous SiO_2 films according to the previously reported procedure[15], a TiO_2 layer was added and the system was calcined to improve the crystallinity of the semiconductor layer. The choice of template size, 30, 60 or 100 nm, proved crucial both from the morphological and electrochemical point of view, as explained in Section 6.2.3. While in the case of bare, non-calcined SiO_2 , the best overall results were obtained with 30 nm PS templates, the most viable amongst these composite films were those prepared using 60 nm nanospheres. Both 30 nm and 100 nm PS latexes led to heterogeneous, cracked coatings; a possible explanation could lie in the thermal treatment these newly prepared films underwent, which may have caused the expansion of the TiO_2 layer, inducing excessive strain and the consequent breakage of the small 30 nm pores. Regarding 100 nm templates, their negative effect on film homogeneity had already been reported in the previous work, thus it is probably inherent to the templates themselves. Previous tests on bare SiO_2 porous films evidenced how the performance optimum was reached when depositing 3 porous layers, a finding that was verified also in this study. In light of these results, further characterizations and tests were carried out exclusively on films

prepared with 60 nm PS nanospheres deposited in three layers, which from now on will be referred as 3SP60T.

Film morphology was investigated via SEM imaging, to verify the homogeneity and porosity of prepared coatings. As can be seen in Figure 6.4, apart from few thicker areas, the film appears homogeneously porous. The microstructure of the film is currently under study through Field Emission SEM.

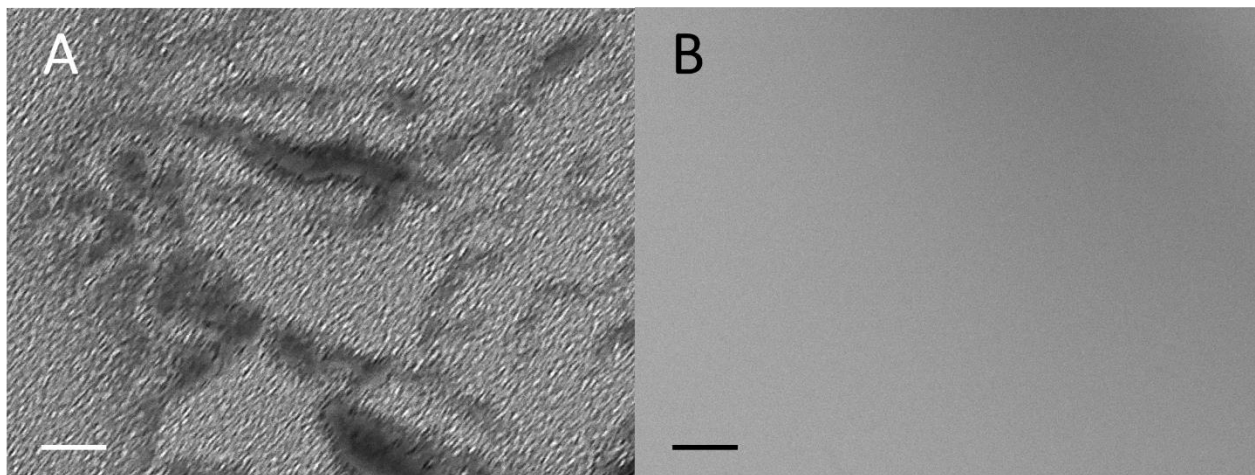


Figure 6.4 - SEM image of sample 3S_P60_T (A) compared to a non-porous TiO₂ film prepared analogously (B). Scale bar is 5 μ m.

6.2.2.2 Slow evaporation TiO₂ films

The second approach to the deposition of TiO₂ films aimed at removing the need of a porous SiO₂ underlayer. The major practical problems that were encountered in preparing porous films made completely of TiO₂ is the intrinsic incompatibility between the alcohol-based titanium isopropoxide sol and the aqueous suspension of polystyrene latex. As Ti(*i*PrO)₄ is extremely susceptible to hydrolysis, leading to the gelation of the solution, their mixture would cause the almost immediate condensation of Ti precursor, making it impossible to deposit a film by spin-coating. To solve this issue, a different water-based precursor solution, containing titanium(IV) bis(ammonium lactato)dihydroxide (TiBALDH), was adopted. Inspired by a previous literature report[17], a new film deposition procedure revolving around the slow evaporation of the precursor solution in presence of PS templates was set up. In this case, 900 nm PS nanospheres were used as hard templates. For the sake of clarity samples prepared at 65°C and 90°C will be referred as TiBALDH_65 and TiBALDH_90 respectively. Contrary to the previous method, this was considerably slower: depending on the operational temperature, it would take between 24 and 48 h to completely cover a sufficiently large glass surface. As shown in Figure 6.5, temperature and evaporation time also had an effect on the morphology of deposited films: lower temperatures, hence longer evaporation times, allowed the PS templates to arrange in a more ordered way. Figure 6.5A depicts the upper section of TiBALDH_65, *i.e.* the first to form after the start of the procedure, while Figure 6.5B portrays the middle section of the same sample, developed when the system was already at regime. As can be noticed, the slow start of the 65°C procedure allowed the polymeric templates to orderly arrange, forming

what appears as a hcp network of pores. This behaviour has been defined as Evaporation-Induced Self-Assembly (EISA)[23] and can allow to control the final structure by adjusting both the chemical parameters, like initial sol composition, pH, aging time, and the processing ones, such as partial vapour pressures, convection, temperature. However, when the systems reaches equilibrium and solvent evaporation is proceeding steadily, PS particles no longer have the time to arrange themselves as neatly as before, leading to the more disordered pore structure of the middle section, which nonetheless seems to be constituted by just two or three layers of pores. On the other hand, when evaporation temperature is increased to 90°C, particle disposition becomes completely random at any stage of the procedure, leading to the disordered pores network represented in Figure 6.5C, where a multitude of randomly overlapping layers of pores can be observed.

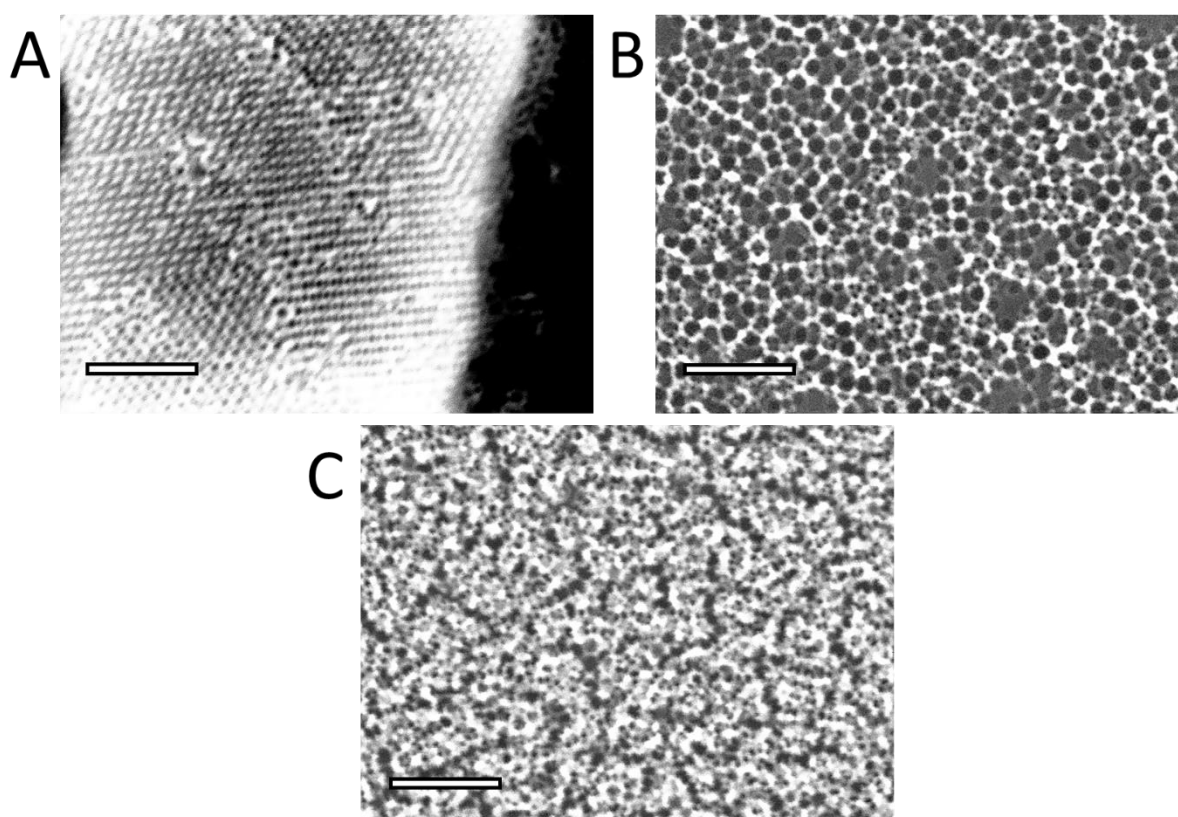


Figure 6.5 - SEM images of films prepared by slow evaporation: two sections of TiBALDH_65 (A, B) and one of TiBALD_90 (C) are shown. Scale bar is 5 μm .

6.2.2.3 Spin-coated TiO_2 films

While interesting for a series of applications, slow evaporation films were considered too thick[17] to be adopted as coatings for sensors. For this reason, a new attempt at depositing pure TiO_2 films by spin-coating was made. As mentioned above, the main problem consisted in the sensitivity of the titanium isopropoxide sol to water. To overcome this issue, a solvent exchange procedure was applied to 200 nm and 350 nm PS latex suspensions, substituting water with ethylene glycol. Although water was not

completely removed from the template suspension, possibly due to the formation of an azeotrope, its amount was reduced enough to guarantee the sol stability for a sufficient time window after mixing, so that it could be deposited by spin-coating on conductive FTO glass.

The effect of three different parameters was investigated: the concentration of TiO_2 sol, the rotational speed and the size of adopted templates. Speaking from a morphological point of view, all prepared films, independently from deposition parameters, showed homogeneous surfaces with a high degree of porosity and good transparency. Figure 6.6 compares SEM images of several coatings, prepared using a highly varied set of parameters. As can be observed, no significant differences can be evidenced by the appearance of films, apart from the slightly larger pores of samples prepared with 350 nm PS templates. The only sample showing a lightly wrinkly surface is TP350_3000_0.17 (Figure 6.6F): this could have possibly been caused by a slightly longer wait from the preparation of the precursor suspension to the deposition procedure, which may have allowed the TiO_2 sol to prematurely begin its gelation process. The number of deposited layers was also tested (Figure 6.7): as before, no morphology changes can be appreciated. The strongest changes concerned film thickness, but their effect could only be evaluated by cyclic voltammetry measurements and will be discussed in Section 6.2.3.

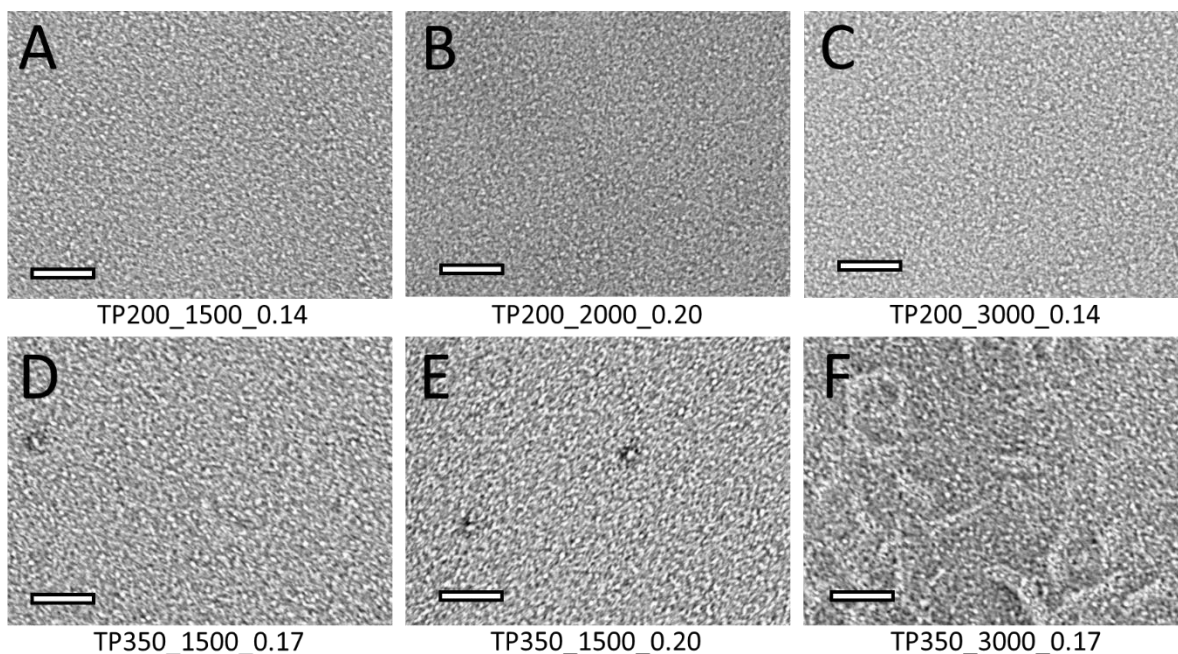


Figure 6.6 - SEM images of spin-coated TiO_2 films prepared by solvent exchange. Scale bar is 5 μm .

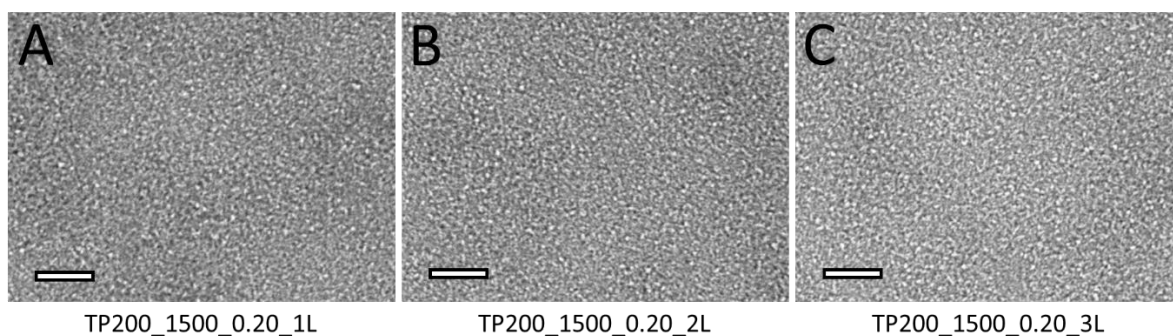


Figure 6.7 - SEM images of samples prepared by depositing 1 layer (A), 2 layer (B) or 3 layers (C). Scale bar is 5 μm .

6.2.3 Electrochemical characterization of TiO_2 films

Since one of the potential applications of these functional TiO_2 films involves their use as anti-fouling, photo-regenerative coatings for sensors, their effect on electrode conductivity was tested by cyclic voltammetry analyses. As mentioned before, samples prepared via slow evaporation resulted too thick to be adopted as electrochemically viable coatings, so this part will only involve films prepared through the other two deposition procedures.

6.2.3.1 $\text{SiO}_2/\text{TiO}_2$ composite films

Figure 6.8 shows the comparison between the cyclic voltammetry curves of sample 3SP60, composed of 3 layers of porous SiO_2 , and sample 3SP60T, which was covered with a TiO_2 layer and calcined. As can be noticed, the $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$ reduction peak current at -0.25 V decreases in the case of sample 3SP60, when only porous silica is present on the FTO electrode, since the electrochemical active area of the conductive glass is covered by an insulating layer. Nonetheless, the registered current intensity is still comparable with that of FTO thanks to the porosity of the SiO_2 layers. These considerations are in agreement with what evidenced by the previous work on these systems[15].

However, with the addition of a TiO_2 layer on top of the SiO_2 ones, the peak current increases greatly and even overcomes that of bare FTO. This phenomenon can be attributed to a combination of two different effects, each contributing to enhance the sensitivity of the underlying electrode to the electrolyte probe: for once, the presence of an additional layer of TiO_2 and subsequent calcination causes the pores to shrink in diameter, increasing the capillary pressure pulling electrolyte molecules towards the FTO surface beneath. At the same time, this effect is further intensified by the greater wettability of the TiO_2 surface compared to the SiO_2 layer, as shown in Figure 6.9. These two phenomena combined generate a strong capillary pull that drives electrolytes to the conductive surface of FTO glass, thus increasing peak currents over those of the pristine electrode itself.

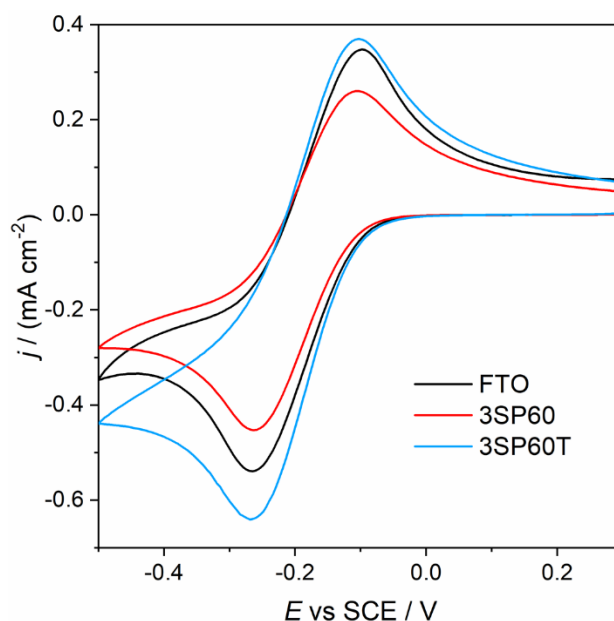


Figure 6.8 - Current peaks comparison between bare FTO, an uncoated SiO₂ porous film (3SP60) and a hybrid SiO₂/TiO₂ film (3SP60T).

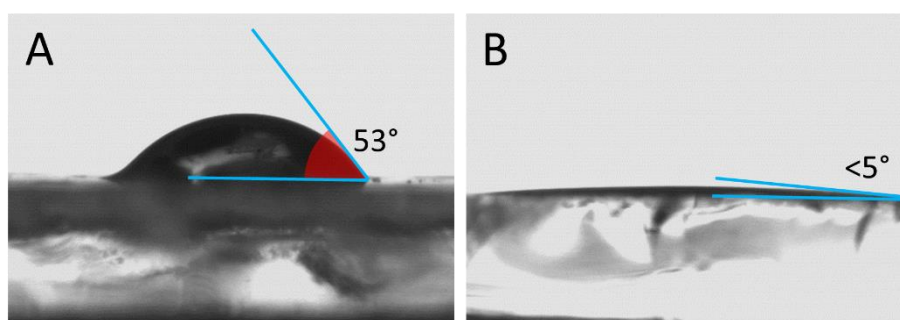


Figure 6.9 - Water contact angle measurements of sample 3SP60 (A) and 3SP60T (B).

6.2.3.2 Spin-coated TiO₂ films

Cyclic voltammetry measurements were carried out on pure TiO₂ spin-coated films as well. Figure 6.10 illustrate the significant influence of deposition parameters on the electrochemical performance of the coatings.

By looking at Figure 6.10A, two different trends may be defined: one concerns the rotational speed of the spin-coater rotor, while the other relates to the concentration of TiO₂ sol. Apparently, for samples prepared with a 0.17 M sol, those deposited at 1500 rpm show a much lower conductivity compared to FTO and even to samples deposited at 3000 rpm. This may possibly be caused by the thinning of the porous coating induced by higher rotational velocities, which would thus result less insulating.

On the other hand, when sol concentration is increased to 0.2 M, the current peak of films deposited at 1500 rpm notably increases, topping even that of bare FTO. This behaviour could be related to the greater amount of TiO₂ precursors, which would further reduce pore size, enhancing the abovementioned capillary pull and consequently improving the electrode conductivity.

Other interesting observations can be made on the effect of layer number, which is portrayed in Figure 6.10B: as can be noticed, increasing the number of successive depositions has a beneficial effect on the electrode conductivity up to an optimum of two layers, after which the addition of more layers seems to negatively affect current intensities. This phenomenon might be related yet again to a change in pore size: the superimposition of porous layers could create a network of smaller pores, as represented in Scheme 6.1, which, as already mentioned, has a positive effect on conductivity. At the same time, an excess of porous layers could obstruct the path of electrolytes, causing a drop in conductivity.

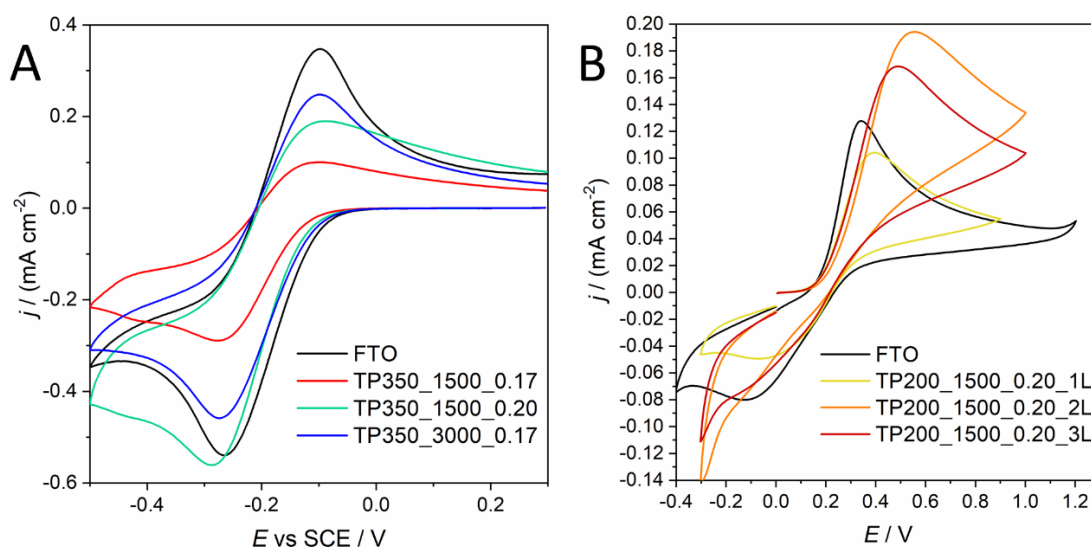
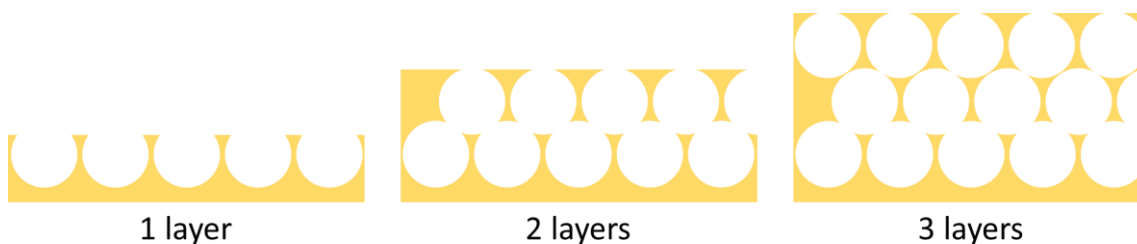


Figure 6.10 - Current peaks comparison between bare FTO, and TiO₂ spin-coated films, prepared with different experimental setups: the effect of rotational speed and sol concentration (A), together with that of deposited layers (B) are reported.



Scheme 6.1 - Schematic representation of the pore shrinking effect induced by multiple depositions.

Overall, the electrochemical properties of deposited TiO₂ films are promising for what concerns their application as sensor coatings, as not only the electrode current intensities remain unaffected by their presence, but in many cases are even improved by their singular morphology and surface properties. Moreover, their behaviour can be finely tuned by acting on many different parameters of the deposition procedure, leaving great room to direct their applicability.

6.2.4 Photo-regeneration tests

One of the most appealing features of TiO_2 porous films is their ability to photocatalytically degrade adsorbed organic compounds, effectively making them self-cleaning surfaces. For this reason, the photo-regeneration capabilities of deposited TiO_2 films were tested by artificially fouling their surface with a long-chained organosilane, then irradiating them with UV light, all while monitoring water contact angle at regular intervals. Figure 6.11 clearly shows that these coatings are remarkably photoactive: before being fouled with the alkylsilane, water contact angle of the TiO_2 surface sits around 20° , while after adsorption of the organic molecule it rises almost to 100° , due to the hydrophobic chains of TEOS. After 60 minutes of light irradiation, contact angle has receded back to the initial values as the surface has been completely cleared from the hydrocarbon chains. All deposited films showed comparable organic degradation times, confirming their role as photo-regenerating coatings. This feature may be applied to periodically regenerate the fouled surface of coated sensors or to cyclically strip the residues of released molecules.

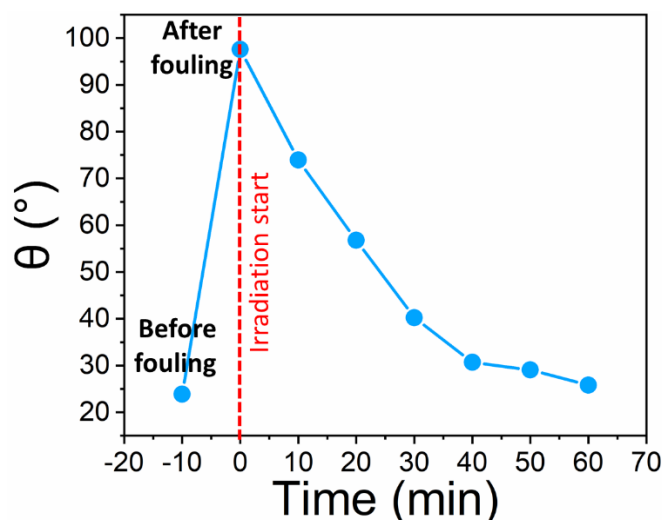


Figure 6.11 - Water contact angle isotherm portraying the wettability changes of TiO_2 surfaces upon artificial fouling with TEOS and after UV irradiation.

6.3 Conclusions

In conclusion, a wide range of functional porous TiO_2 coatings was developed, finely tuning their morphology through the use of differently sized polystyrene templates, multiple deposition techniques and, within each of them, a great number of distinct parameters. Their effect on the electrochemical properties of underlying conductive surfaces was evaluated via cyclic voltammetry measurements, which revealed that in most cases the addition of a porous TiO_2 film enhanced the intrinsic current intensities of the FTO support. This behaviour could be explained on the basis of capillary pressure

and surface energy, as the implementation of a porous, highly hydrophilic layer would drive electrolytes to the sensor surface beneath by means of capillary pull forces.

The photo-regeneration capabilities of deposited films were tested by purposely fouling them with hydrophobic organic molecules and monitoring the change in water contact angle after UV light irradiation. All of them demonstrated to be apt to efficiently remove organic fouling elements adsorbed on their surface within a relatively short time window. These results display the great potential of these porous TiO₂ films as size-exclusion photo-regenerative sensor coatings.

Future work will be aimed at characterizing the film thickness and at preparing porous TiO₂ films by spin coating using PS templates of smaller size.

6.4 References

- [1] M.D. Stamate, On the dielectric properties of dc magnetron TiO₂ thin films, *Appl. Surf. Sci.* 218 (2003) 318–323. doi:10.1016/S0169-4332(03)00624-X.
- [2] D.J. Kim, S.H. Hahn, S.H. Oh, E.J. Kim, Influence of calcination temperature on structural and optical properties of TiO₂ thin films prepared by sol–gel dip coating, *Mater. Lett.* 57 (2002) 355–360. doi:10.1016/S0167-577X(02)00790-5.
- [3] J. Dewalque, R. Cloots, F. Mathis, O. Dubreuil, N. Krins, C. Henrist, TiO₂ multilayer thick films (up to 4 µm) with ordered mesoporosity: influence of template on the film mesostructure and use as high efficiency photoelectrode in DSSCs, *J. Mater. Chem.* 21 (2011) 7356. doi:10.1039/c1jm10288e.
- [4] X.-N. Wang, W. Tang, A. Gordon, H.-Y. Wang, L. Xu, P. Li, B. Li, Porous TiO₂ Film Immobilized with Gold Nanoparticles for Dual-Polarity SALDI MS Detection and Imaging, *ACS Appl. Mater. Interfaces.* 12 (2020) 42567–42575. doi:10.1021/acsami.0c12949.
- [5] T. Brezesinski, J. Wang, J. Polleux, B. Dunn, S.H. Tolbert, Templated Nanocrystal-Based Porous TiO₂ Films for Next-Generation Electrochemical Capacitors, *J. Am. Chem. Soc.* 131 (2009) 1802–1809. doi:10.1021/ja8057309.
- [6] I. KIM, A. ROTHSCILD, D. YANG, H. TULLER, Macroporous TiO₂ thin film gas sensors obtained using colloidal templates, *Sensors Actuators B Chem.* 130 (2008) 9–13. doi:10.1016/j.snb.2007.07.092.
- [7] G. Soliveri, V. Pifferi, G. Panzarasa, S. Ardizzzone, G. Cappelletti, D. Meroni, K. Sparnacci, L. Falciola, Self-cleaning properties in engineered sensors for dopamine electroanalytical detection, *Analyst.* 140 (2015) 1486–1494. doi:10.1039/C4AN02219J.
- [8] V. Pifferi, G. Soliveri, G. Panzarasa, S. Ardizzzone, G. Cappelletti, D. Meroni, L. Falciola, Electrochemical sensors cleaned by light: a proof of concept for on site applications towards integrated monitoring systems, *RSC Adv.* 5 (2015) 71210–71214. doi:10.1039/C5RA12219H.
- [9] V. Pifferi, G. Soliveri, G. Panzarasa, G. Cappelletti, D. Meroni, L. Falciola, Photo-

renewable electroanalytical sensor for neurotransmitters detection in body fluid mimics, *Anal. Bioanal. Chem.* 408 (2016) 7339–7349. doi:10.1007/s00216-016-9539-3.

- [10] J. Chapman, F. Regan, Nanofunctionalized Superhydrophobic Antifouling Coatings for Environmental Sensor Applications-Advancing Deployment with Answers from Nature, *Adv. Eng. Mater.* 14 (2012) B175–B184. doi:10.1002/adem.201180037.
- [11] L. Delauney, C. Compère, M. Lehaitre, Biofouling protection for marine environmental sensors, *Ocean Sci.* 6 (2010) 503–511. doi:10.5194/os-6-503-2010.
- [12] S. Chandra, A.D. Miller, D.K.Y. Wong, Evaluation of physically small p-phenylacetate-modified carbon electrodes against fouling during dopamine detection in vivo, *Electrochim. Acta.* 101 (2013) 225–231. doi:10.1016/j.electacta.2012.11.022.
- [13] W. Harreither, R. Trouillon, P. Poulin, W. Neri, A.G. Ewing, G. Safina, Cysteine residues reduce the severity of dopamine electrochemical fouling, *Electrochim. Acta.* 210 (2016) 622–629. doi:10.1016/j.electacta.2016.05.124.
- [14] K.H. Chae, Y.M. Jang, Y.H. Kim, O.-J. Sohn, J. Il Rhee, Anti-fouling epoxy coatings for optical biosensor application based on phosphorylcholine, *Sensors Actuators B Chem.* 124 (2007) 153–160. doi:10.1016/j.snb.2006.12.012.
- [15] L. Rimoldi, V. Pifferi, D. Meroni, G. Soliveri, S. Ardizzone, L. Falciola, Three-dimensional mesoporous silica networks with improved diffusion and interference-abating properties for electrochemical sensing, *Electrochim. Acta.* 291 (2018) 73–83. doi:10.1016/j.electacta.2018.08.131.
- [16] J. Procházka, L. Kavan, V. Shklover, M. Zukalová, O. Frank, M. Kalbáč, A. Zukal, H. Pelouchová, P. Janda, K. Mocek, M. Klementová, D. Carbone, Multilayer Films from Templated TiO₂ and Structural Changes during their Thermal Treatment, *Chem. Mater.* 20 (2008) 2985–2993. doi:10.1021/cm071452e.
- [17] B. Hatton, L. Mishchenko, S. Davis, K.H. Sandhage, J. Aizenberg, Assembly of large-area, highly ordered, crack-free inverse opal films, *Proc. Natl. Acad. Sci.* 107 (2010) 10354–10359. doi:10.1073/pnas.1000954107.
- [18] J.W. Goodwin, J. Hearn, C.C. Ho, R.H. Ottewill, Studies on the preparation and characterisation of monodisperse polystyrene latices, *Colloid Polym. Sci.* 252 (1974) 464–471. doi:10.1007/BF01554752.
- [19] A. Antonello, G. Soliveri, D. Meroni, G. Cappelletti, S. Ardizzone, Photocatalytic remediation of indoor pollution by transparent TiO₂ films, *Catal. Today.* 230 (2014) 35–40. doi:10.1016/j.cattod.2013.12.033.
- [20] S. Ardizzone, C.L. Bianchi, G. Cappelletti, S. Gialanella, C. Pirola, V. Ragaini, Tailored Anatase/Brookite Nanocrystalline TiO₂. The Optimal Particle Features for Liquid- and Gas-Phase Photocatalytic Reactions, *J. Phys. Chem. C.* 111 (2007) 13222–13231. doi:10.1021/jp0741096.
- [21] B. Trouiller, R. Reliene, A. Westbrook, P. Solaimani, R.H. Schiestl, Titanium Dioxide Nanoparticles Induce DNA Damage and Genetic Instability In vivo in Mice, *Cancer Res.* 69 (2009) 8784–8789. doi:10.1158/0008-5472.CAN-09-2496.
- [22] J.W. Goodwin, J. Hearn, C.C. Ho, R.H. Ottewill, Studies on the preparation and characterization of monodisperse polystyrene latices, *Colloid Polym. Sci.* 252 (1974)

464–471.

- [23] D. Grosso, F. Cagnol, G.J. de A.A. Soler-Illia, E.L. Crepaldi, H. Amenitsch, A. Brunet-Bruneau, A. Bourgeois, C. Sanchez, Fundamentals of Mesostructuring Through Evaporation-Induced Self-Assembly, *Adv. Funct. Mater.* 14 (2004) 309–322. doi:10.1002/adfm.200305036.

CHAPTER 7

Functionalization of porous TiO₂ films for the development of nanostructured antimicrobial coatings

The TiO₂ porous films with tailored morphology discussed in the previous chapter proved very promising as functional coatings, even if their surface was not modified with any active molecule. To further explore the possibilities provided by these systems, functionalization of their surface with bioactive molecules bound by a stimuli-responsive linker complex was investigated.

A field that has undoubtedly gained a lot of traction in the last decade is the development of antimicrobial coatings for the applications such as smart food packaging and sanitary surfaces. In this respect, it should be noticed that one of the major causes of food spoilage is microbiological contamination which causes the alteration of the product healthiness, nutritional and sensory features. The consumption of contaminated food can lead to several diseases: the world health organization 2015 report estimated the occurrence of about 600 million food-born illness cases per year and of 420 000 associated deaths[1]. In this context, antimicrobial materials are of great interest for the food industry. Released antimicrobial substances can be either synthetic or natural compounds: for consumption safety, most of research is focused on natural substances[2]. Plant essential oils (EOs) are natural compounds often characterized by antimicrobial activity which could represent a natural non-toxic alternative to chemical preservatives[1].

Among all EOs, cinnamaldehyde (CIN) has very appealing properties. CIN is a phenolic terpenoid and the major constituent of cinnamon bark oil and was classified as GRAS (General Recognized As Safe) by the FDA (Food and Drug Administration)[3]. The industry utilizes cinnamaldehyde in innumerable products, including, but not limited to, non-alcoholic beverages, ice cream, candy, baked goods, chewing gum, condiments and meats at levels ranging from 9 to 4900 ppm[4]. It is a low-cost and highly safe compound, characterized by a characteristic, pleasant aroma[5]. Due to its excellent antibacterial, antifungal, anti-inflammatory, anti-cancer activity and antioxidant properties, CIN is widely adopted in the food, cosmetic, biomedical and pharmaceutical industries[3,6]. For example, in the biomedical field, cinnamaldehyde was adopted for wound dressing applications[6,7] and in drug delivery systems for cancer treatments[8–11]. Minimum inhibitory concentrations (MICs) of CIN for bacteria range from 22.89 mg/L for *Listeria monocytogenes* in vapor phase, to 4.2 mg/L for *Escherichia coli* and *Salmonella typhimurium* in broth dilution. For fungi, the vapor phase range is from 0.52 mg/L for *Penicillium notatum*, to 22.89 mg/L for *Aspergillus flavus*, to 4.62 mg/L for *Candida albicans*[12]. However, CIN is characterized by high volatility, poor water solubility, instability and can be easily oxidized upon exposure to light, oxygen and

heat, which limit its antibacterial efficiency and hinder its application in food, agriculture and other fields[5,6,13]. Recently, the encapsulation of CIN in solid particles or film systems has been proposed to increase its bioavailability and physical stability and decrease its volatility[5,13].

Nonetheless, most of the literature reports make use of tailored polymer systems for the diffusion-based slow release of CIN[1,3–7,9,10,12,13], whereas the use of a purposely functionalized oxidic support could open up the possibility for a stimuli-induced release, while also improving its stability, thus increasing the shelf-life. As it is well known that spoilage of dairy products involves an initial acidification step[14] and that the growth of several fungi and moulds is favoured by acidic pH[15,16], a pH-sensitive chemical system analogous to what was showed in Chapter 2 was applied here to modify the surface of TiO₂ films. APTES was thus chosen as a linker and grafted on TiO₂ porous films: in this way, the terminal amine group of the silane (-NH₂) was available to perform a condensation reaction with the aldehydic group of CIN (-HC=O), leading to the formation of an imine bond (-HC=N-) between APTES and CIN.

As the chemical characterization of films is generally more challenging compared to powders, commercial TiO₂ nanoparticles were adopted as models to monitor the functionalization procedure after each step.

The stability of the APTES-CIN imine bond is sensitive to environmental pH, as its hydrolysis is catalysed at low pH, thus enabling a pH-controlled release of CIN under acidic conditions. To assess the efficacy of these pH-responsive devices, the release of surface-grafted cinnamaldehyde was monitored at different pH values. The photocatalytic nature of the TiO₂ support could then be exploited to regenerate the exhausted antimicrobial surface by removing any organic residue through brief UV light irradiation.

7.1 Experimental procedures

7.1.1 Materials

Chemicals were acquired from Sigma-Aldrich (unless differently stated) and were used without further purification. Water was doubly distilled and deionised through a Milli-Q apparatus.

7.1.2 Synthesis of polystyrene templates

Polystyrene latices were synthesized analogously to what reported in Chapter 6. Briefly, 0.45 mM K₂S₂O₈ was dissolved in 100 mL of the chosen solvent (either water or ethylene glycol) in a 500 mL two-neck round-bottom flask equipped with a water-cooled reflux condenser, under nitrogen atmosphere and magnetic stirring. The flask was put in an oil bath, the stirrer was adjusted to 400 rpm and then styrene (0.53 M) was added dropwise. The polymerization reaction was heated for 24 h at 95 °C and then gradually cooled to room temperature. The obtained latices had a final concentration of 3.9%w in water and 5.7%w in ethylene glycol.

7.1.3 TiO₂ film deposition

Before film deposition, the borosilicate glass supports were cleaned by subsequent washings with acetone, 2-propanol and Milli-Q water in an ultrasound bath. Substrates were then immersed for 30 s in concentrated sulfuric acid (98%), rinsed with Milli-Q water and dried in the oven at 150 °C. Water-suspended 200 nm polystyrene nanoparticles (60 µL) were mixed with particles suspended in ethylene glycol (240 µL). Then 500 µL of titania sol was added to the mixture and deposited on a 2 x 2 cm² borosilicate glass substrate. Film deposition was performed by spin coating a single layer, using a spinning rate of 1500 rpm for 20 s, with an acceleration of 500 rpm/s. Films were then calcined at 400 °C for 4 hours under O₂ flux (9 NL h⁻¹).

7.1.4 Film functionalization with cinnamaldehyde

The TiO₂ film functionalization with APTES was performed according to a previously reported procedure[17]. A titania porous film was immersed in 20 mL of anhydrous toluene inside a jacketed reactor at 65°C under N₂. After 1 h, 400 µL of APTES was added to the solution. The reaction was stopped after 3 h at 65 °C, the film was retrieved and washed briefly with fresh toluene in an ultrasound bath, before being dried under a N₂ flux.

The functionalized TiO₂-APTES film was then placed in a jacketed reactor at 65°C equipped with a reflux condenser and a drying CaCl₂ tube: 20 mL of anhydrous methanol was added while bubbling N₂. After 1 h, 80 µL of cinnamaldehyde was added while bubbling N₂. After 3 h, the TiO₂-CIN film was retrieved.

7.1.5 TiO₂ particles functionalization

As model, commercial TiO₂ powder (Evonik P25), referred as TiO₂p, were functionalized with APTES and cinnamaldehyde. Powder functionalization was carried out in a jacketed reactor at 65°C. 500 mg of TiO₂p was added to 50 mL of anhydrous toluene under N₂. The suspension was magnetically stirred for 1 h, then 1 mL of APTES was added. The suspension was kept under stirring at 65 °C for 3 h. At the end, functionalized powders were collected by centrifugation, washed four times with toluene and dried in a ventilated oven at 70 °C for 24 h. The retrieved sample was labelled TiO₂p-APTES.

Before grafting cinnamaldehyde, TiO₂p-APTES was dried in a ventilated oven at 70 °C overnight, to remove physisorbed water. The reaction was carried out in anhydrous conditions: a jacketed reactor at 65°C was equipped with a reflux condenser and a drying CaCl₂ tube. While bubbling N₂, 400 mg of TiO₂p-APTES was added to 50 mL of anhydrous methanol. The suspension was kept under magnetic stirring for 1 h, then 201 µL of cinnamaldehyde were added while bubbling N₂. After 3 h, the product was retrieved by centrifugation, washed five times with methanol and dried in a ventilated oven at 70 °C for 24 h. The obtained sample was labelled TiO₂p-CIN.

7.1.6 Release tests

Release tests were carried out by placing the TiO₂-CIN film in 5 mL of either Milli-Q water (pH 5.5) or phosphate buffer (pH 7.4) while magnetically stirring. The solution was sampled every 30 min: each time, few mL was withdrawn and analysed with a UV-vis spectrophotometer ($\lambda = 280$ nm), before being added back to the main solution. All operations were carried out in the dark to avoid any photoinduced effect.

7.1.7 Photo-regeneration tests

Similarly to the tests performed in the previous chapter, regeneration tests were carried out on film substrates by purposely fouling their surface with long alkyl chain moieties, followed by regeneration by UV light irradiation. Film fouling was carried out by chemical vapor deposition of 100 μ L of triethoxy(octyl)silane, performed according to a previously reported procedure[18]. Films were then UV-irradiated with a Jelosil HG500 lamp (effective irradiation power: 30 mW cm⁻²). The fouling and surface regeneration were monitored via static contact angle measurements, performed on a Krüss Easydrop instrument using ultrapure water droplets of 8 μ L.

7.1.8 Sample characterization

Fourier Transform Infrared (FTIR) spectra were registered using a Perkin Elmer Spectrum 100 spectrometer in ATR mode, acquiring 24 scans between 4000 and 400 cm⁻¹ with a resolution of 4.0 cm⁻¹.

Thermogravimetric Analysis (TGA) curves were acquired using a Mettler-Toledo TGA/DSC 3+ STAR System, between 30 and 900 °C with a rate of 3 °C min⁻¹. TGA analyses were conducted under air flux.

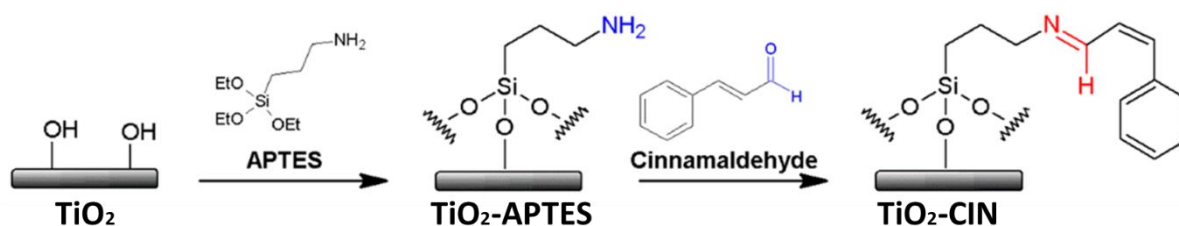
Elemental CHN analyses were carried out on a CHN Perkin-Elmer 2400 analyzer.

Contact angle measurements were performed using a Krüss EasyDrop instrument and software. For each film, five static angles were measured, using 2 μ L droplets of water.

UV-vis absorption spectra of the solutions were collected with a Shimadzu UV-2600 spectrophotometer, scanning wavelengths between 200 and 500 nm.

7.2 Results and discussion

To investigate the potential use of these porous TiO_2 films as functional coatings capable of releasing bioactive molecules under certain environmental conditions, spin-coated TiO_2 films prepared with 200 nm PS templates, were loaded with cinnamaldehyde. Similarly to what was reported in Chapter 2 in the case of HNT-7TH, APTES was adopted to form an imine bond with the formyl group of cinnamaldehyde, as illustrated in Scheme 7.1, to exploit its sensitivity to pH variations. Interestingly, grafted cinnamaldehyde was stable for months after the functionalization, proving the beneficial effect of immobilization on a TiO_2 support, which prevented molecule degradation.



Scheme 7.1 - Schematic representation of cinnamaldehyde grafting on TiO_2 surfaces.

7.2.1 Characterization of model TiO_2 powders

The $\text{TiO}_2\text{p-APTES}$ and $\text{TiO}_2\text{p-CIN}$ grafted powders were characterized in depth to confirm the formation of the imine bond ($\text{C}=\text{N}$) and quantify the functionalization and grafting degree.

7.2.1.1 FTIR spectroscopy

Figure 7.1 shows the FT-IR spectra of $\text{TiO}_2\text{p-APTES}$ and $\text{TiO}_2\text{p-CIN}$ powders in comparison with pristine TiO_2p . The $\text{TiO}_2\text{p-APTES}$ spectrum presents the characteristic bands at 2926 cm^{-1} (C-H_x stretching), 1563 cm^{-1} (NH_2 scissoring) and 1025 cm^{-1} (Si-O-Si stretching), ascribable to APTES grafted on the oxide surface. In the $\text{TiO}_2\text{p-CIN}$ sample, the formation of the $\text{C}=\text{N}$ double bond is highlighted by the peak at 1635 cm^{-1} , which is absent in the cinnamaldehyde FT-IR spectrum (Figure 7.2).

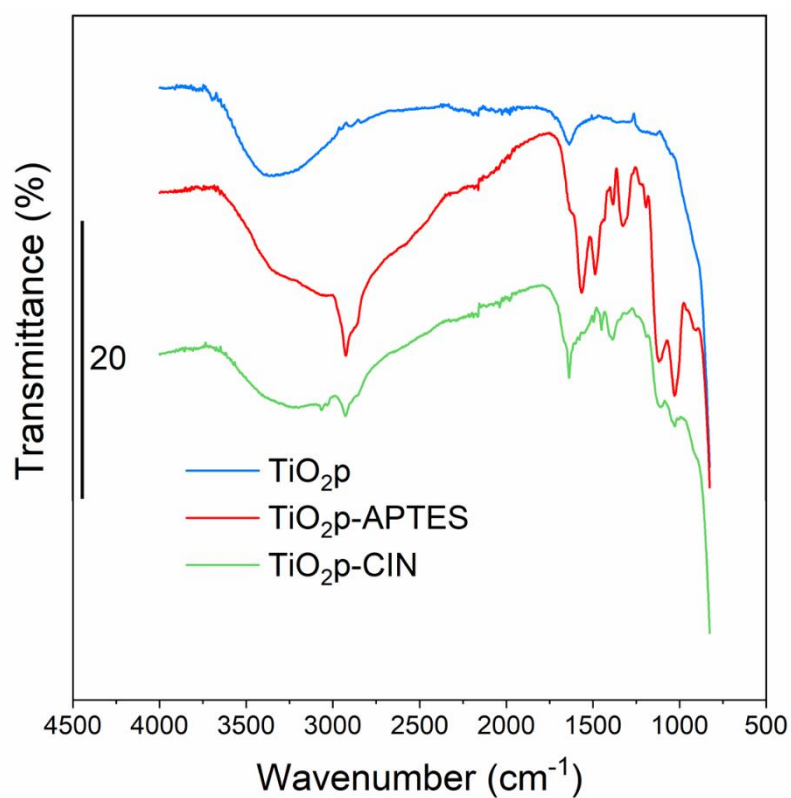


Figure 7.1 - FT-IR spectra of TiO₂p, TiO₂p-APTES and TiO₂p-CIN.

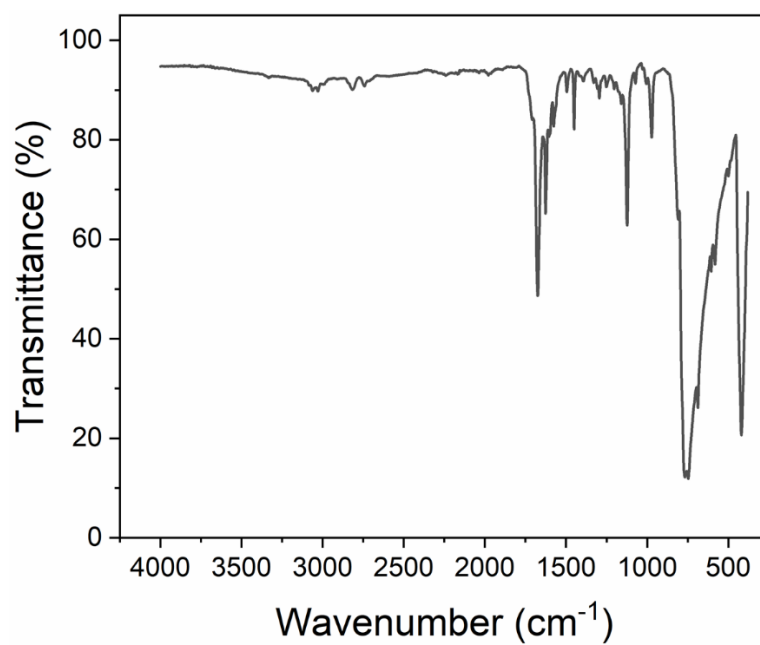


Figure 7.2 - FT-IR spectrum of CIN.

7.2.1.2 Thermogravimetric analysis

The TGA reported in Figure 7.3 allowed the quantification of the functionalization degree and the loading percentage of, respectively, TiO₂p-APTES and TiO₂p-CIN samples. Notwithstanding the fundamental morphological differences, especially concerning specific surface area, between powders and porous films, it was still considered beneficial to gauge the loading efficiency on a TiO₂ substrate. Moreover, TGA data was later elaborated to provide surface elemental ratios, certainly more fitting for the characterization of films. The TiO₂p reference sample showed a weight loss of about 3%, the majority of which occurred under 200 °C, ascribable to the loss of oxide surface hydration. The functionalized TiO₂p-APTES powder follows the TG curve of bare TiO₂p at low temperatures, showing a lower degree of surface hydration. At higher temperatures, above 280°C, TiO₂p-APTES is characterized by a marked weight loss (around 3%): this weight loss can be related to the degradation of the organic chain of APTES. The TiO₂p-CIN powders are characterized by the same behavior at low temperatures, showing an even lower surface hydration below 200 °C and a 3% weight loss between 280 and 450 °C. At even higher temperatures, the CIN grafted particles show a further drop in weight (3%) between 450 and 500 °C, possibly due to the thermal degradation of the cinnamaldehyde aromatic ring. It is thus possible to estimate the functionalization degree of APTES in TiO₂p-APTES and TiO₂p-CIN, as well as the grafting surface density of CIN in TiO₂p-CIN, analogously to what was presented in Chapter 3:

The moles of thermally degraded APTES in TiO₂p-APTES were obtained from the weight loss starting at 280 °C (0.03%) in TG curves according to the following equation:

$$n_{APTES} = \frac{0.03 \text{ g}_{APTES}}{58 \text{ g mol}^{-1}} = 0.52 \text{ mmol}$$

where 58 g mol⁻¹ is the molecular weight of APTES alkyl chain (C₃H₈N). It should be noted that the ethoxide groups of APTES molecule were considered completely hydrolyzed on the basis of previous works[19] and on grounds of CHN analyses.

The mass of oxide present in 1 g of TiO₂p-APTES was calculated as follows:

$$m_{TiO_2} = 1 - (n_{APTES} \cdot 120 \text{ g mol}^{-1} + 0.01 \text{ g}_{H_2O}) = 0.93 \text{ g}$$

where 120 g mol⁻¹ is the molecular weight of the attached APTES (C₃H₈NSiO₃), assuming all the ethoxide groups were lost, and 0.01 g corresponds to the mass loss in the TG curve due to water loss.

Thus, by considering the specific surface area (SSA) of the adopted TiO₂ powder (50 m²g⁻¹), the surface density of APTES, δ_{APTES} , in TiO₂p-APTES sample was calculated:

$$\delta_{APTES} = \frac{n_{APTES} \cdot N_A}{m_{TiO_2} \cdot SSA_{TiO_2}} \cdot 10^{-18} \frac{nm^2}{m^2} = 6.9 \frac{molecules}{nm^2}$$

where N_A is the Avogadro's number ($6.022 \cdot 10^{23}$ molecules mol^{-1}).

From the TG analysis of TiO_2 p-CIN, the surface coverage of both APTES and CIN were determined. In this sample, two main weight losses are observed, both accounting for 3% w/w: the first one could be attributed to the degradation of aliphatic chains and the second ascribable to the thermal decomposition of the aromatic ring of CIN. From the latter, the moles of grafted CIN in 1 g of TiO_2 p-CIN were estimated according to the following equation:

$$n_{CIN} = \frac{0.03 g_{C_6H_5}}{77 g mol^{-1}} = 0.39 mmol$$

where $77 g mol^{-1}$ corresponds to the molar mass of the aromatic ring.

The moles of APTES in 1 g of TiO_2 p-CIN could then be determined from the initial weight loss, taking into account the alkyl chain in grafted CIN molecules:

$$n_{APTES} = \frac{0.03 g_{C_6H_9N} - (n_{CIN} \cdot 39 g mol^{-1})}{58 g mol^{-1}} = 0.26 mmol$$

where 39 and $58 g mol^{-1}$ are the molecular mass of the aliphatic moiety of grafted CIN (C_3H_9) and the alkyl chain of covalently bonded APTES (C_3H_9N), respectively.

The mass of oxide in 1 g of TiO_2 p-CIN was calculated as follows:

$$m_{TiO_2} = 1 g - (n_{APTES} \cdot 120 g mol^{-1} + n_{CIN} \cdot 116 g mol^{-1} + 0.005 g_{H_2O}) = 0.92$$

where $120 g mol^{-1}$ is the molecular weight of the attached APTES ($C_3H_8NSiO_3$), assuming all the ethoxide groups were lost, $116 g mol^{-1}$ is the molecular weight of the grafted CIN (C_9H_8) and 0.005 g corresponds to the mass loss due to water loss in the TG curve.

The surface densities of APTES moieties and CIN molecules in TiO_2 p-CIN were calculated according to the following equations:

$$\delta_{APTES} = \frac{n_{APTES} \cdot N_A}{m_{TiO_2} \cdot SSA_{TiO_2}} \cdot 10^{-18} \frac{nm^2}{m^2} = 3.5 \frac{molecules}{nm^2}$$

$$\delta_{CIN} = \frac{n_{CIN} \cdot N_A}{m_{TiO_2} \cdot SSA_{TiO_2}} \cdot 10^{-18} \frac{nm^2}{m^2} = 5.1 \frac{molecules}{nm^2}$$

The results show a δ_{APTES} in TiO_2p -APTES and TiO_2p -CIN of 6.9 molecules/nm² and 3.5 molecules/nm² respectively. The observed decrease in the functionalization degree of APTES is ascribable to the loss of physisorbed APTES molecules during the preparation of TiO_2p -CIN in methanol. The value of 3.5 molecules/nm² is that of a complete monolayer of APTES molecules as reported in previous works[20]. The loading degree of CIN is of 5.1 molecules/nm², which shows a small excess of CIN molecules with respect to APTES: this can be explained with the presence of not only chemically bonded CIN molecules, but also a fraction of physisorbed CIN.

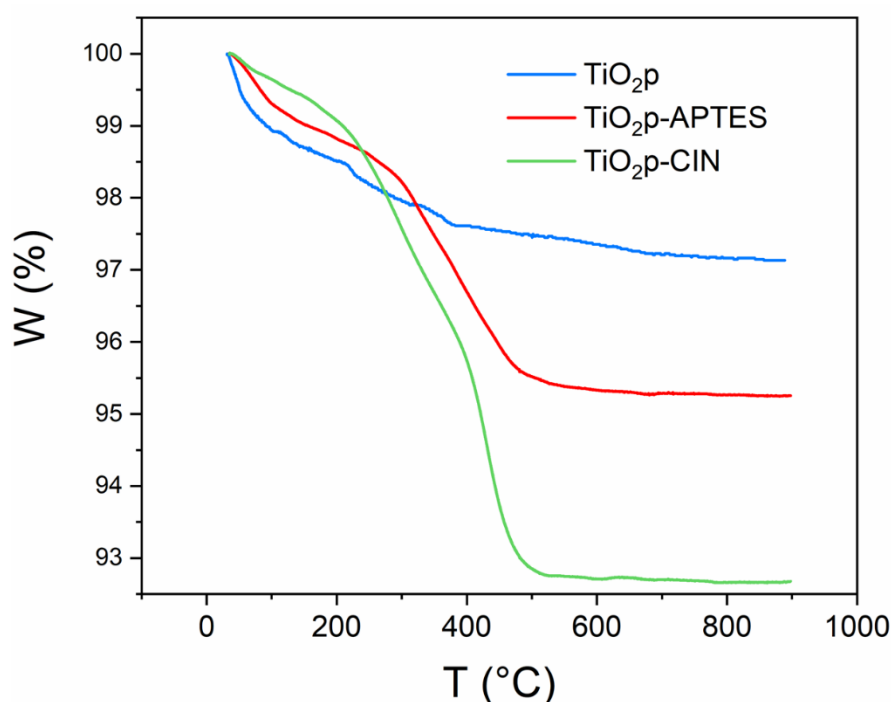


Figure 7.3 - Thermogravimetric analyses of TiO_2p , TiO_2p -APTES and TiO_2p -CIN

7.2.1.3 CHN analysis

Another way to determine the loading degree of the functionalized powders is through CHN analyses. The measurements were carried out on bare oxide, TiO_2p -APTES and TiO_2p -CIN samples: the results are reported in Table 7.1.

Sample	%C	%H	%N
TiO ₂ p	0.3 2	0.5 6	0.0 6
TiO ₂ p- APTES	3.2 3	1.3 0	1.1 1
TiO ₂ p-CIN	5.5 0	0.6 4	0.4 3

Table 7.1 - CHN measurements of bare oxide compared to the functionalized powders.

It is noteworthy that, in TiO₂p-APTES sample, the ratio between the moles of C and N is 3.4. This experimental value is very close to the one of an APTES molecule which has lost all three ethoxy groups (theoretical ratio $\text{mol}_C/\text{mol}_N = 3$). Thus, APTES molecules can be considered completely condensated to the oxide surface. With this assumption, it is possible to evaluate a theoretical C/N molar ratio for the APTES-CIN molecule ($\text{C}_{12}\text{H}_{16}\text{NSiO}_3$): $\text{mol}_C/\text{mol}_N = 12$. The experimental value, obtained from the TiO₂p-CIN sample, is slightly larger than the theoretical one: $\text{mol}_C/\text{mol}_N = 14.9$. From these results it is possible to assume that nearly all the amine groups of APTES reacted to cinnamaldehyde molecules.

Furthermore, from CHN measurements, it is possible to evaluate the functionalization degree and loading density of APTES and CIN:

The δ_{APTES} in TiO₂p-APTES was determined starting from the amount of N determined by CHN analyses (1.11%). The moles of APTES in 1 g of TiO₂p-APTES were determined according to the equation:

$$n_{\text{APTES}} = n_N = \frac{1.11 \text{ g}_N}{14 \text{ g mol}^{-1} \cdot 100} = 0.793 \text{ mmol}$$

where 14 g mol^{-1} is the atomic mass of N.

The total amount of oxide in 1 g of TiO₂p-APTES was determined as follows:

$$m_{\text{Si in } 100 \text{ g TiO}_2\text{p} - \text{APTES}} = n_{\text{APTES}} \cdot 28 \text{ g mol}^{-1} \cdot 100 = 2.22 \text{ g}$$

$$m_{\text{TiO}_2} = \frac{100 \text{ g} - (3.23 \text{ g} + 1.30 \text{ g} + 1.11 \text{ g} + m_{\text{Si}})}{100} = 0.92 \text{ g}$$

where 28 g mol^{-1} is the atomic mass of Si, 3.23 g and 1.30 g are the mass of C and H, respectively, in 100 g of TiO₂p-APTES (Tab.1).

The surface density of APTES moieties was thus determined as follows:

$$\delta_{APTES} = \frac{n_{APTES} \cdot N_A}{m_{TiO_2} \cdot SSA_{TiO_2}} \cdot 10^{-18} \frac{nm^2}{m^2} = 10.4 \frac{molecules}{nm^2}$$

The surface densities of APTES and CIN in TiO₂p-CIN were also estimated from CHN analyses. The moles of APTES were determined from N content (0.43%, Tab. 1) according to the following equation:

$$n_{APTES} = n_N = \frac{0.43 \text{ g}_N}{14 \text{ g mol}^{-1} \cdot 100} = 0.307 \text{ mmol}$$

The CIN amount was determined from C content (5.50%), taking into account the fraction of carbon due to APTES molecules. In this respect, the ethoxide groups were considered fully hydrolyzed, as stated previously. The moles of CIN in 1 g of TiO₂p-CIN was calculated according to the following equation:

$$n_{CIN} = \frac{n_{C_{tot}} - n_{C_{APTES}}}{9} = \left[\frac{5.50 \text{ g}_C}{12 \text{ g mol}^{-1} \cdot 100} - (3 \cdot n_{APTES}) \right] \cdot \frac{1}{9} = 0.407 \text{ mmol}$$

where 12 g mol⁻¹ is the atomic mass of carbon and 9 is the number of carbon atoms in a CIN molecule.

The mass of the oxide was then calculated as follows:

$$m_{Si} = n_{APTES} \cdot 28 \text{ g mol}^{-1} \cdot 100 = 0.86 \text{ g}$$

$$m_{TiO_2} = \frac{100 \text{ g} - (5.50 \text{ g} + 0.64 \text{ g} + 0.43 \text{ g} + m_{Si})}{100} = 0.93 \text{ g}$$

where 0.64 g is the mass of H in 100 g of TO-APTES (Tab.1).

The surface densities of APTES moieties and CIN molecules in TiO₂p-CIN were calculated according to the following equations in TiO₂p-CIN sample:

$$\delta_{APTES} = \frac{n_{APTES} \cdot N_A}{m_{TiO_2} \cdot SSA_{TiO_2}} \cdot 10^{-18} \frac{nm^2}{m^2} = 4.0 \frac{molecules}{nm^2}$$

$$\delta_{CIN} = \frac{n_{CIN} \cdot N_A}{m_{TiO_2} \cdot SSA_{TiO_2}} \cdot 10^{-18} \frac{nm^2}{m^2} = 5.3 \frac{molecules}{nm^2}$$

The obtain functionalization degree of APTES in TiO₂p-APTES sample is 10.4 molecules/nm², which is slightly higher than the one obtained from TGA measurements and thus relates to an APTES multilayer. Meanwhile, δ_{APTES} in TiO₂p-CIN sample resulted to be of 4.0 molecules/nm²: as in the TG analyses, we observe a loss of physisorbed APTES molecules in the CIN grafted sample, and the remaining 4.0 molecules/nm² probably correspond to a complete APTES monolayer[20]. The δ_{CIN} in TiO₂p-APTES-CIN powders was estimated to be 5.3 molecules/nm², which is nearly the same obtained from the TGA analyses and, as in the TGA, shows a small excess of CIN molecules, probably due to the presence of a physisorbed fraction of the aldehydes.

7.2.2 Cinnamaldehyde release tests

The release of surface grafted cinnamaldehyde was monitored at different pH values through UV spectroscopy. The results are shown in Figure 7.4. The difference between the release rates at pH 5.5 and pH 7.4 is blatant: while at physiological conditions the release of cinnamaldehyde is very limited, a shift to a more acidic pH leads to a significant increase in the concentration of cinnamaldehyde released in solution, mirroring the behaviour of the device based on halloysite. These preliminary results represent an encouraging step towards the preparation of stimuli-responsive coatings acting as antimicrobial functional layers in food packaging or other instances susceptible to biological fouling.

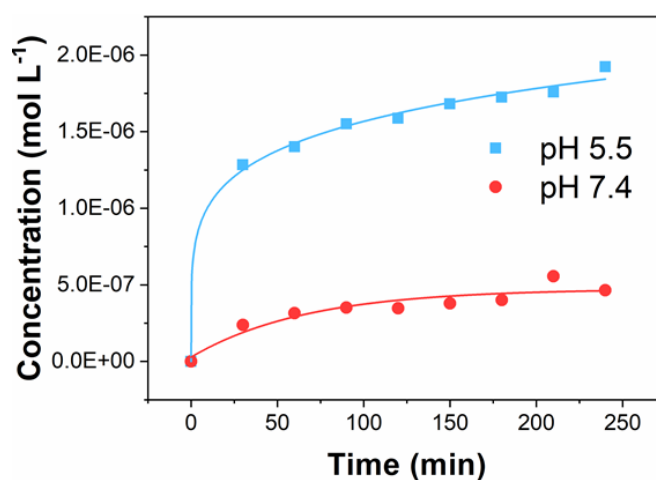


Figure 7.4 - Comparison between the release rate of cinnamaldehyde from a functionalized 2x2 cm² TP200 film at pH 5.5 and pH 7.4.

7.2.3 Photo-regeneration tests

Photo-regeneration was tested on TiO₂-CIN samples, by measuring the static water contact angle before and after light irradiation: after being irradiated with UV light, samples were successfully cleared of any organic residues within 45 minutes. This

feature may be applied to periodically to cyclically strip the residues of released molecules, as the APTES-cinnamaldehyde system, and re-functionalize it as needed.

7.3 Conclusions

For the first time, the use of a TiO₂-based material for the encapsulation and stimuli-controlled release of cinnamaldehyde has been investigated. Surface functionalization with the stimuli-responsive APTES-CIN imine system was successfully performed and proved to selectively release the bioactive molecule under a certain pH threshold. The photo-regeneration capabilities of functionalized films were tested by irradiating them with UV light, which eliminated any trace of organic compounds on the surface within minutes. This feature appears particularly useful to extend the life of these coatings: after use, they could be simply regenerated by UV light and functionalised again to operate as before. These results display the potential of these porous TiO₂ films to be used as biocompatible antimicrobial surfaces for food packaging. Beside the food industry, also agriculture may benefit from such devices, as natural pesticides based on essential oils could be loaded onto porous biocompatible tablets and dispersed where needed, to fight infesting species. In this respect, the use of EISA films presented in Chapter 6, which exhibited higher surface area but lower photocatalytic properties, could possibly grant higher amounts of released cinnamaldehyde at the cost of slower regeneration rates.

7.4 References

- [1] G. Granata, S. Stracquadanio, M. Leonardi, E. Napoli, G.M.L. Consoli, V. Cafiso, S. Stefani, C. Geraci, Essential oils encapsulated in polymer-based nanocapsules as potential candidates for application in food preservation, *Food Chem.* 269 (2018) 286–292. doi:10.1016/j.foodchem.2018.06.140.
- [2] C.-Y. Hu, M. Chen, Z.-W. Wang, Release of Thymol, Cinnamaldehyde and Vanillin from Soy Protein Isolate Films into Olive Oil, *Packag. Technol. Sci.* 25 (2012) 97–106. doi:10.1002/pts.964.
- [3] J. Muller, A. Casado Quesada, C. González-Martínez, A. Chiralt, Antimicrobial properties and release of cinnamaldehyde in bilayer films based on polylactic acid (PLA) and starch, *Eur. Polym. J.* 96 (2017) 316–325. doi:10.1016/j.eurpolymj.2017.09.009.
- [4] A. Nostro, R. Scaffaro, M. D'Arrigo, L. Botta, A. Filocamo, A. Marino, G. Bisignano, Study on carvacrol and cinnamaldehyde polymeric films: Mechanical properties, release kinetics and antibacterial and antibiofilm activities, *Appl. Microbiol. Biotechnol.* 96 (2012) 1029–1038. doi:10.1007/s00253-012-4091-3.

- [5] M. Ji, X. Sun, X. Guo, W. Zhu, J. Wu, L. Chen, J. Wang, M. Chen, C. Cheng, Q. Zhang, Green synthesis, characterization and in vitro release of cinnamaldehyde/sodium alginate/chitosan nanoparticles, *Food Hydrocoll.* 90 (2019) 515–522. doi:10.1016/j.foodhyd.2018.12.027.
- [6] Y. Liu, X. Liang, R. Zhang, W. Lan, W. Qin, Fabrication of electrospun polylactic acid/Cinnamaldehyde/ β -cyclodextrin fibers as an antimicrobial wound dressing, *Polymers (Basel)*. 9 (2017) 1–14. doi:10.3390/polym9100464.
- [7] I. Liakos, L. Rizzello, D.J. Scurr, P.P. Pompa, I.S. Bayer, A. Athanassiou, All-natural composite wound dressing films of essential oils encapsulated in sodium alginate with antimicrobial properties, *Int. J. Pharm.* 463 (2014) 137–145. doi:10.1016/j.ijpharm.2013.10.046.
- [8] J. Noh, B. Kwon, E. Han, M. Park, W. Yang, W. Cho, W. Yoo, G. Khang, D. Lee, Amplification of oxidative stress by a dual stimuli-responsive hybrid drug enhances cancer cell death, *Nat. Commun.* 6 (2015). doi:10.1038/ncomms7907.
- [9] W. Yoo, D. Yoo, E. Hong, E. Jung, Y. Go, S.V.B. Singh, G. Khang, D. Lee, Acid-activatable oxidative stress-inducing polysaccharide nanoparticles for anticancer therapy, *J. Control. Release.* 269 (2018) 235–244. doi:10.1016/j.jconrel.2017.11.023.
- [10] B. Kim, E. Lee, Y. Kim, S. Park, G. Khang, D. Lee, Dual acid-responsive micelle-forming anticancer polymers as new anticancer therapeutics, *Adv. Funct. Mater.* 23 (2013) 5091–5097. doi:10.1002/adfm.201300871.
- [11] I.L. Liakos, C. Menager, N. Guigo, A.M. Holban, F. Iordache, F. Pignatelli, A.M. Grumezescu, B. Mazzolai, N. Sbirrazzuoli, Suberin/ trans-Cinnamaldehyde Oil Nanoparticles with Antimicrobial Activity and Anticancer Properties When Loaded with Paclitaxel, *ACS Appl. Bio Mater.* 2 (2019) 3484–3497. doi:10.1021/acsabm.9b00408.
- [12] M.P. Balaguer, M. Borne, P. Chalier, N. Gontard, M.H. Morel, S. Peyron, R. Gavara, P. Hernandez-Munoz, Retention and release of cinnamaldehyde from wheat protein matrices, *Biomacromolecules*. 14 (2013) 1493–1502. doi:10.1021/bm400158t.
- [13] R. Cui, K. Jiang, M. Yuan, J. Cao, L. Lin, Z. Tang, Y. Qin, Antimicrobial film based on polylactic acid and carbon nanotube for controlled cinnamaldehyde release, *J. Mater. Res. Technol.* 9 (2020) 10130–10138. doi:10.1016/j.jmrt.2020.07.016.
- [14] F. Ebrahimi Tirtashi, M. Moradi, H. Tajik, M. Forough, P. Ezati, B. Kuswandi, Cellulose/chitosan pH-responsive indicator incorporated with carrot anthocyanins for intelligent food packaging, *Int. J. Biol. Macromol.* 136 (2019) 920–926. doi:10.1016/j.ijbiomac.2019.06.148.
- [15] M.A. Gock, A.D. Hocking, J.I. Pitt, P.G. Poulos, Influence of temperature, water activity and pH on growth of some xerophilic fungi, *Int. J. Food Microbiol.* 81 (2003) 11–19. doi:10.1016/S0168-1605(02)00166-6.
- [16] S. Rawat, Food Spoilage: Microorganisms and their prevention, *Asian J. Plant Sci. Res.* 5 (2015) 47–56.
- [17] G. Soliveri, V. Pifferi, G. Panzarasa, S. Ardizzone, G. Cappelletti, D. Meroni, K. Sparnacci, L. Falciola, Self-cleaning properties in engineered sensors for dopamine electroanalytical detection, *Analyst*. 140 (2015) 1486–1494. doi:10.1039/C4AN02219J.

- [18] A. Antonello, G. Soliveri, D. Meroni, G. Cappelletti, S. Ardizzone, Photocatalytic remediation of indoor pollution by transparent TiO₂ films, *Catal. Today*. 230 (2014) 35–40. doi:10.1016/j.cattod.2013.12.033.
- [19] T. Taroni, S. Cauteruccio, R. Vago, S. Franchi, N. Barbero, E. Licandro, S. Ardizzone, D. Meroni, Thiahelicene-grafted halloysite nanotubes: Characterization, biological studies and pH triggered release, *Appl. Surf. Sci.* 520 (2020) 146351. doi:10.1016/j.apsusc.2020.146351.
- [20] D. Meroni, L. Lo Presti, G. Di Liberto, M. Ceotto, R.G. Acres, K.C. Prince, R. Bellani, G. Soliveri, S. Ardizzone, A Close Look at the Structure of the TiO₂ -APTES Interface in Hybrid Nanomaterials and Its Degradation Pathway: An Experimental and Theoretical Study, *J. Phys. Chem. C*. 121 (2017) 430–440. doi:10.1021/acs.jpcc.6b10720.

PART III

Superparamagnetic iron oxide nanoparticles

INTRODUCTION

Being amongst the most abundant materials in the Earth's crust, iron oxides have always been employed for many purposes because of their physicochemical properties, especially the magnetic features displayed by some members of this group, such as magnetite and maghemite. Although during the twentieth century these materials were adopted mostly in the large-scale production of consumer goods, like permanent magnets or videotape, in the last twenty years they have found new life in many high-tech application fields in the form of nanoparticles. More precisely, the development and diffusion of superparamagnetic iron oxide nanoparticles (SPION) has generated an ever-growing interest towards their application in a huge number of instances, from the production of high-efficiency data storage devices, to the design of diagnostic and therapeutic nanosystems for the treatment of invasive illnesses.

III.1 GENERAL PROPERTIES

III.1.1 Structural properties

Out of all the numerous species of iron oxide, hematite, or $\alpha\text{-Fe}_2\text{O}_3$, is the most abundant, due to its high thermodynamic stability. Its unit cell is hexagonal, defined by the hcp packing of anions (Figure III.1A)[1]. However, the phases that get the most attention for the synthesis of SPION are its polymorph maghemite ($\gamma\text{-Fe}_2\text{O}_3$) and especially magnetite (Fe_3O_4). The first can exhibit both a spinel and inverse spinel crystal structures, in which cation vacancies guarantee the neutrality of the unit cell charge (Figure III.1B). For each cell there are 2 and 1/3 vacant sites which can be either structurally ordered, leading to a spinel structure, or randomly distributed, in which case the oxide arranges as an inverse spinel. Due to entropic factors, the second case represents the general occurrence of maghemite[2,3]. As $\gamma\text{-Fe}_2\text{O}_3$ shows very little absorbance above 700 nm, it appears brown-orange in colour[4]. Magnetite is the allotropic form of maghemite, as these two iron oxides are isomorphous from a crystallographic perspective (Figure III.1C). Both phases present an inverse spinel face-

centred cubic oxide lattice with almost identical unit cell dimensions, with lattice parameters of $a = 8.39 \text{ \AA}$ for Fe_3O_4 and either $a = 8.34 \text{ \AA}$ or $8.35 \text{ \AA}$ for $\gamma\text{-Fe}_2\text{O}_3$ depending on how it generated[5,6], making it very difficult to tell them apart using X-ray powder diffraction alone[7]. What distinguishes them is the oxidation state of iron cations, as $\gamma\text{-Fe}_2\text{O}_3$ has only Fe^{3+} ions like hematite, whereas in Fe_3O_4 2/3 of the iron sites are Fe^{3+} and 1/3 are Fe^{2+} . Magnetite features an inverse spinel crystal structure ($Fd\bar{3}m$), where ferric ions are equally distributed between tetrahedral and octahedral sites, expressed in the chemical formula $[\text{Fe}^{3+}]_A [\text{Fe}^{3+}, \text{Fe}^{2+}]_B \text{O}^{2-}_4$, where A represent the tetrahedral sites and B the octahedral ones. The $\text{Fe}^{2+}\text{--Fe}^{3+}$ intervalence charge transfer causes absorption throughout all UV-vis and IR regions of the electromagnetic spectrum, thus granting magnetite a characteristic black colour[7]. When put in aqueous conditions or oxygen-containing environments, magnetite can slowly oxidise to the all- Fe^{3+} maghemite. However, oxidation proceeds a lot slower when it is stored as a powder at room temperature[4,8].

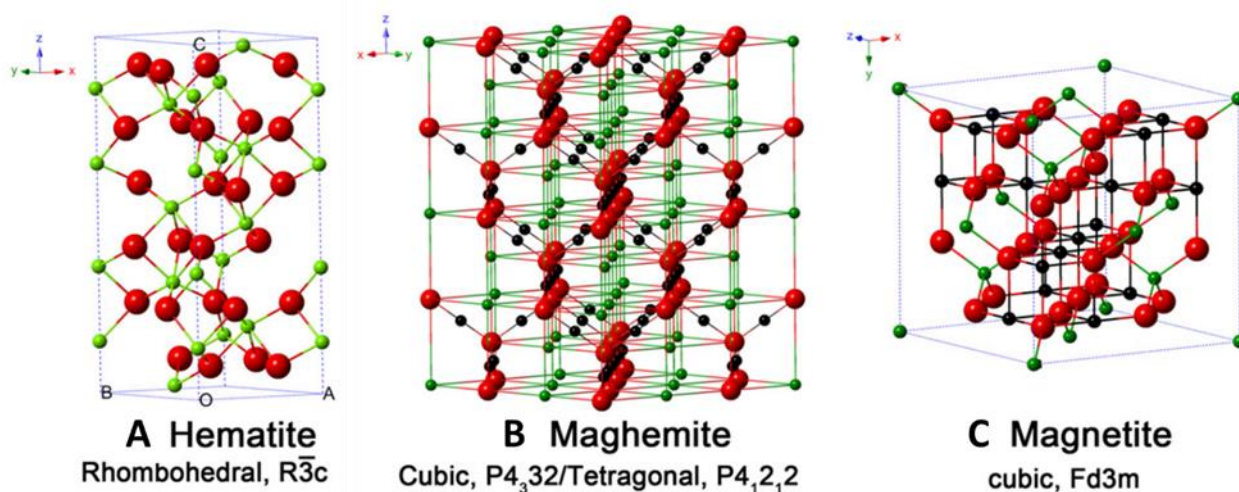


Figure III.1 - Crystal structure and crystallographic data of the hematite (A), magnetite (B) and maghemite (C). The black spheres are Fe^{2+} , the green ones are Fe^{3+} and the red ones are O^{2-} [9].

III.1.2Magnetic properties

The three mentioned iron oxides all display different forms of magnetism. Hematite is antiferromagnetic below the Morin transition at 250 K ($-23 \text{ }^\circ\text{C}$), and a canted antiferromagnet or weakly ferromagnetic above the Morin transition and below its Néel temperature at 948 K ($675 \text{ }^\circ\text{C}$), above which it is paramagnetic[10](Figure III.2A). It shows an extremely feeble net magnetization. Maghemite exhibits a ferromagnetic behaviour (Figure III.2B), with a net magnetic moment of 2-3 μB per formula unit and a relatively high Curie temperature of 890 K. Together with its chemical stability and low cost, this has led to its wide application as magnetic pigment in electronic recording media since the late 1940's[11]. However, amongst the abovementioned phases,

magnetite is the most sought after in materials science: in its natural state, it is ferrimagnetic (Figure III.2C), showing a higher net magnetization compared to maghemite, measuring 4-5 μB per formula unit, and a similar Curie temperature of 860 K. Its greater magnetic saturation and the presence of Fe^{2+} ions, responsible of triggering reactive oxygen species (ROS) production through the Fenton reaction, make it the first choice when developing nanosized iron oxide magnetic materials for biomedical applications[12]. Both ferromagnetic and ferrimagnetic oxides, when placed within an external magnetic field, get magnetized and keep a net magnetization even after its removal, because of the irreversible alignment of magnetic moments in their sublattices. As a result, their magnetization moment shows a hysteresis loop as a function of the external field. However, both magnetite and maghemite exhibit a superparamagnetic behaviour when their size reaches below 20 nm. This means that they are only magnetised under the influence of an external magnetic field, with zero coercivity, thus a reversal of field is not required to reduce their magnetization to zero[13] as there is no hysteresis. This is because at less than 20 nm, each particle acts as a single magnetic domain and can be easily reoriented by an energy $\approx k_{\text{B}}T$ (where T is close to room temperature). Furthermore, even the shape of iron oxide nanoparticles can greatly affect their magnetic properties: it was recently demonstrated that the extremely high surface-to-volume ratio of ultrathin iron oxide nanowhiskers measuring 2-20 nm leads to strong paramagnetic signals[14].

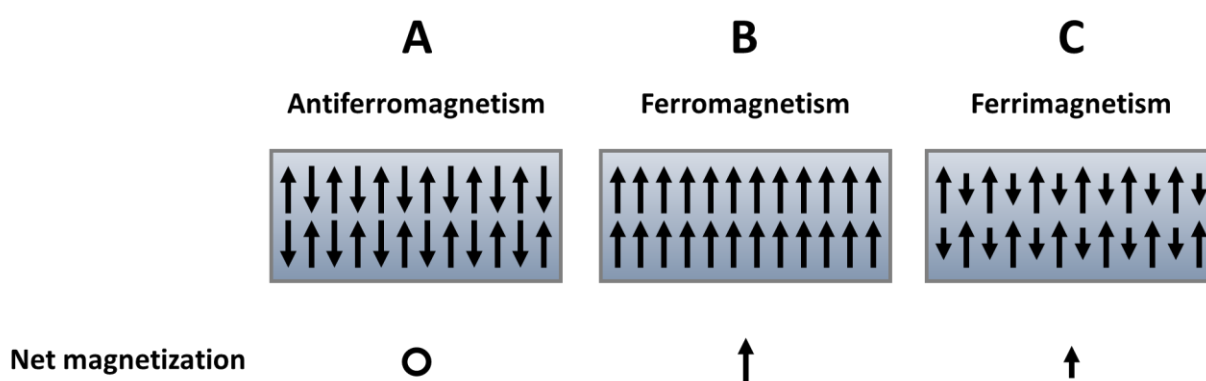


Figure III.2 - Types of magnetization induced by different ordering of spins.

III.2 APPLICATIONS

Within the last two decades, the synthesis of superparamagnetic nanoparticles has been intensively researched for numerous technological applications: just to name a few, they have been studied for catalysis[15], energy and data storage[16–19], enzyme immobilisation[20], as low-cost magnetically separable adsorbents in wastewater treatment[21] and separation processes[22,23]. Still, the field that has possibly bet the most on these materials is that of nanomedicine, where SPION are studied and employed in a variety of applications, which include hyperthermia, targeted drug delivery and MRI signal enhancement.

III.2.1 Hyperthermia

One of the most interesting applications of the superparamagnetic nature of small iron oxide nanoparticles is magnetic hyperthermia. First envisaged in the seminal work of Jordan et al. in 1993[24], it employs an alternating magnetic field (AMF) to induce rapid oscillations in the magnetic moment of SPION. The friction generated by this high-frequency reorientation releases heat to the surrounding environment, following the so-called Brownian and Néel relaxation processes. This phenomenon can be exploited *in vivo* to increase the temperature of tumour tissue and destroy nearby pathological cells. It can be either used in direct oncological treatments or as an adjuvant in conjunction with chemotherapy and radiotherapy. While this technique is not exclusive to superparamagnetic materials, under the same conditions ferromagnetic systems generate less heat because of the higher hysteresis components of multiple-domain particles. The heat generated by the process can usually raise the temperature of surrounding cells up to 42-45°C, which is more than enough to induce apoptosis, activating numerous degrading pathways that lead to the disruption of tumour cells[25]. This procedure is made even more interesting by the fact that usually cancer cells, which exhibit multiple somatic mutations, are more sensitive to temperature increases and begin the apoptotic process at around 43°C, conditions that are still bearable by healthy cells[26]. Generally, the SPION needed to induce hyperthermia can be administered in two main ways: they can either be injected topically around the tumour area or be infused intravenously. The latter could provide a more homogeneous distribution in the entire organism, but it requires targeting features, for example specific antibodies, to induce a significant accumulation of SPION not only around tumour cells but also within their membranes. In this way, upon activation of the magnetic oscillator, the released thermal energy would be limited to the tumour and its very close surroundings, thus collateral damage would only affect few healthy cells[27,28]. In addition to the already cited positive effects of hyperthermia, it has been recently reported that this treatment enhances the expression of Heat Shock Proteins (HSP), which are linked to higher tumour immunogenicity[26].

In the last few years, studies on hyperthermia-based therapies have intensified, leading to some interesting results: it has been recently demonstrated that cancer tissue is sought and infiltrated by mesenchymal stem cells (MSC)[29], which inspired the study of a therapeutic agent combining MSC and SPION through endocytosis. These modified stem cells are able to penetrate inside tumour tissue, where they can be heated up through a magnetic field. Once the outer MSC envelope has been disrupted, SPION are quickly released and internalized by cancer cells, which greatly strengthens the ablative effect of hyperthermia. Through all this, the properties of MSC are not affected by the incorporation of SPION[30]. As mentioned previously, both preclinical and clinical data show that hyperthermia is not only feasible but also very effective in combination with radiation therapy: a study performed on 112 patients suffering of the tumour *glioblastoma multiformae* demonstrated that survival is doubled when γ therapy is combined with hyperthermia[31]. Another enticing possibility was presented in a most recent study featuring the *in situ* synthesis of SPION into cancer cells: tumour tissue was treated with a mixture of FeCl₂ and zinc gluconate, which reacted within the cell membrane producing ZnO nanoparticles and SPION. Appropriate *in vitro* studies revealed that reactive oxygen species, especially H₂O₂, and the glycolytic pathway had a

significant role in the biosynthesis of the nanoparticles. Exposure of treated cells to an alternating magnetic field for 30 minutes resulted in a substantial temperature rise of 5–6 °C, confirming the great potential of this innovative technique[32].

III.2.2 Drug delivery

The topic of targeted therapies was already introduced earlier in this thesis: conventional chemotherapy almost always produces serious damage to the whole organism, not just to the targeted area. It is thus crucial to develop highly selective therapeutics, designed to seek and treat cancer cells. Such specificity should also increase the healing power of treatments by allowing the administration of higher drug doses, as they would not be limited by the concern for their effect on healthy tissues. In this regard, SPION are being intensively studied as drug carriers because of their unique magnetic properties, which can be exploited to direct and concentrate them to the selected location through the use of an external magnetic field[33].

In the literature there are various examples of SPION-based drug carriers: Alexiou et al.[34] reported the use of iron oxide nanoparticles covered by starch derivatives modified with phosphate groups, which were employed to bind mitoxantrone, as innovative chemotherapeutics. The study showed that a strong magnetic field gradient localized around the tumour area induced the accumulation of loaded SPION, which was proved to happen both between and within cancer cells by electron microscopy investigations. Another study illustrated the development of a biologically stable methotrexate-immobilized SPION-based drug carrier that may potentially be used for real-time monitoring of drug delivery through magnetic resonance imaging[35]. Methotrexate (MTX) was grafted on the surface of magnetic nanoparticles via a poly(ethylene glycol) self-assembled monolayer (PEG-SAM). Cellular uptake experiments showed that the uptake of NP-PEG-MTX conjugates by glioma cells was considerably higher than that of control nanoparticles. Magnetic resonance imaging in 9L rat glioma cells cultured with NP-PEG-MTX of various concentrations showed significant contrast enhancement. Another example involves Cisplatin: despite being a popular chemotherapeutic agent used in the treatment of solid tumours, Cisplatin causes heavy collateral damage to healthy tissues. Part of the problem is also represented by its low water solubility and lipophilicity, as well as the fact that cancer cells can become resistant to its action. To solve the problems presented by the clinical administration of Cisplatin, an innovative porous and hollow SPION carrier was synthesised through the controlled oxidation of surface Fe ions followed by acid digestion[36]. After being loaded with Cisplatin, these systems proved stable under physiological conditions, significantly slowing down drug release rates. At lower pH, however, the pores opened, allowing for their content to diffuse much faster, a behaviour that could be exploited to delay drug release up until cellular intake through lysosomal internalization. Hollow SPION were functionalized with the antibody Herceptin to target breast cancer SK-BR-3 cells, so that their IC₅₀ reached as low as 2.9 µM, much lower than the 6.8 µM needed for free Cisplatin. An additional advantage presented by this approach consists in the protection of Cisplatin from plasma proteins, which are prevented from deactivating it before it reaches its target. Speaking of antibodies, they have been adopted in multiple

studies on oncological treatments due to their ability to target specific patterns in the tertiary structure of proteins. One of these works was presented by Yang et al.[37]: to avoid the inhibition of the superparamagnetic properties of iron oxide nanoparticles that can sometimes occur upon surface modification, they developed a novel drug delivery system by encapsulating SPION within cationic liposomes and engineered their surface with antitransferrin receptor single-chain antibody fragments. This approach allowed these nanocomposites to selectively target overexpressed transferrin receptors on tumour cells.

III.2.3 Magnetic Resonance Imaging contrast agents

Magnetic Resonance Imaging (MRI) is among the most relevant non-invasive imaging techniques available today, both for clinical diagnosis and research purposes. Its ever-increasing popularity is certainly due to the fact that it produces high-resolution anatomical images of living subjects, other than allowing the monitoring of several metabolic and physiological parameters, including tissue perfusion and heart function. When used as a diagnostic technique, MRI measures water proton relaxation: a strong static magnetic field is applied to the subject, in order to align the hydrogen nuclear spin along its direction[38]. A radio frequency (RF) pulse is then emitted to excite the spins and shift their orientation to perpendicular to the applied field direction. Said pulse must have a specific resonant frequency, called Larmor's frequency. After removing the RF pulse, excited spins tend to naturally relax back to the equilibrium state, aligning themselves to the external field. Return to equilibrium happens via two independent but simultaneous processes, called T1 and T2 relaxation. In the case of T1 relaxation (also called longitudinal or spin-lattice), excited spins realign to the external field direction, while for T2 relaxation (also called transverse or spin-spin), their perpendicular moments lose coherence and dephase to zero. Relaxation times for both T1 and T2 can be influenced by their surrounding environment, allowing the instrument to produce images based on the differences between analysed tissues. The resolution of MRI is greatly enhanced by the administration of contrast agents[39], which improve image contrast by shortening the longitudinal (T1) and transverse (T2) relaxation times of water protons. In this sense, SPION are commonly used as cell tracking agents through MRI[40–42]. They enhance contrast by altering the transverse relaxation time of protons contained within surrounding tissue. Beyond medical diagnosis, MRI has been heavily explored for disease therapy and monitoring, such as imaging-guided drug delivery[43–46], cell-based therapy[47,48], angiography and atherosclerotic plaque imaging[49], and pH monitoring[50].

For these reasons, many research works have focused on the development and application of MRI contrast agents based on SPION, not only capable of improving image resolution, but also of targeting and selectively binding to specific tissues, to further simplify the identification of pathological conditions. To this purpose, conjugating SPION with specific ligands, such as antibodies, peptides, polysaccharides, nucleotides, aptamers and other synthetic mimics can generate a variety of targeted, vectorized contrast agents. In theory, this would offer a virtually unlimited number of possibilities for the imaging of tumours[51]. One of the most promising approach is the conjugation of SPION and antibodies. A fine example of targeted imaging based on

antibodies was illustrated in a 2016 study on the use of mesothelin-functionalised SPION against pancreatic carcinoma[52], which is one of the deadliest tumours in urgent need of a satisfying therapy. Mesothelin is a protein, the membrane form thereof was confirmed to be associated with some tumour types, such as pancreatic adenocarcinoma but also ovarian cancer or mesothelioma[53]. The study focused on the synthesis of SPION coupled with anti-mesothelin antibodies and their effectiveness in targeting pancreatic carcinoma cells in mouse xenografts. Results were encouraging, as no relevant cytotoxicity was observed whereas the system displayed a high specificity for the target, making it a viable candidate for MRI contrast enhancement. Another example of targeted imaging was reported by Boutry et al.[54], who adopted a superparamagnetic iron oxide contrast agent obtained by grafting a synthetic mimetic of sialyl Lewisx (sLex), a natural ligand of the endothelial inflammatory adhesion molecule E-selectin expressed in leukocytes, on the dextran coating of ultrasmall SPION (USPION). This device was able to target cells involved in an inflammatory response both *in vitro*, on cultured human umbilical vein endothelial cells (HUVECs) stimulated to express inflammatory adhesion molecules, and *in vivo* on a mouse model of hepatitis. These results confirmed that this system is thus well-suited for the MRI diagnosis of inflammation and the *in vitro* evaluation of endothelial cell activation.

Clinically speaking, T1-enhanced images are preferred when aiming for better resolution and easy detection[55], whereas T2-enhanced images are useful for the examination of inflamed areas or fluid-rich structures. Therefore, the development of T1/T2 bimodal contrast agents has recently attracted much attention[56–58], as it could increase the accuracy of the diagnostic process by improving both detection limits while also retaining higher scan resolution. Since it has been recently demonstrated that SPION larger than 10 nm normally act as T2 contrast agents, while particles smaller than 4 nm behave instead as T1 contrast agents[38,59], nanoparticles exhibiting a size range between 5 and 10 nm could be engineered through surface and magnetization control to perform T1/T2 imaging[60]. In this framework, several research works explored the preparation of T1/T2 SPION-based contrast agents. Gao et al.[61], demonstrated the capability of 5.4 nm PEG-coated iron oxide nanoparticles in the enhancement of both T1 and T2: the T1-weighted images of an aqueous nanoparticle solution showed positive enhancement, while T2-weighted images exhibited negative contrast, suggesting their potential as both positive and negative contrast agents. In another study, it was demonstrated that even lower size nanoparticles could act as dual contrast agents: Lu et al.[62] injected 3.3 nm USPION in mice, collecting both T1 and T2-weighted scans of their liver and kidneys, and found out that the administered contrast agent provided T1 enhancement compared to the control sample while also greatly reducing T2 in T2-weighted images, indicating that those nanosystems were also effective T2 contrast agents. These preliminary studies confirm the attractiveness of optimally sized SPION as dual T1/T2 MRI contrast agents.

In light of these considerations, the next Chapter will revolve around the synthesis of properly sized SPION with the aim of using them for biomedical purposes, some of which have been discussed above. Among others, their use as MRI contrast agents in particular will be treated more in detail, due to the ubiquity and relevance of this diagnostic technique.

References

- [1] A.W. McReynolds, T. Riste, Magnetic Neutron Diffraction from Fe₃O₄, *Phys. Rev.* 95 (1954) 1161–1167. doi:10.1103/PhysRev.95.1161.
- [2] T.D. Glotch, G.R. Rossman, Mid-infrared reflectance spectra and optical constants of six iron oxide/oxyhydroxide phases, *Icarus*. 204 (2009) 663–671. doi:10.1016/j.icarus.2009.07.024.
- [3] C. Pecharroman, T. Gonzalez-Carreno, J.E. Iglesias, The infrared dielectric properties of maghemite, γ -Fe₂O₃, from reflectance measurement on pressed powders, *Phys. Chem. Miner.* 22 (1995). doi:10.1007/BF00202677.
- [4] J. Tang, M. Myers, K.A. Bosnick, L.E. Brus, Magnetite Fe₃O₄ Nanocrystals: Spectroscopic Observation of Aqueous Oxidation Kinetics †, *J. Phys. Chem. B.* 107 (2003) 7501–7506. doi:10.1021/jp027048e.
- [5] J.A. Cuenca, K. Bugler, S. Taylor, D. Morgan, P. Williams, J. Bauer, A. Porch, Study of the magnetite to maghemite transition using microwave permittivity and permeability measurements, *J. Phys. Condens. Matter.* 28 (2016) 106002. doi:10.1088/0953-8984/28/10/106002.
- [6] C.J. Goss, Saturation magnetisation, coercivity and lattice parameter changes in the system Fe₃O₄- γ -Fe₂O₃, and their relationship to structure, *Phys. Chem. Miner.* 16 (1988). doi:10.1007/BF00203200.
- [7] R.M. Cornell, U. Schwertmann, *The Iron Oxides*, Wiley, 2003. doi:10.1002/3527602097.
- [8] I. David, A.J.E. Welch, The oxidation of magnetite and related spinels. Constitution of gamma ferric oxide, *Trans. Faraday Soc.* 52 (1956) 1642. doi:10.1039/tf9565201642.
- [9] W. Wu, Z. Wu, T. Yu, C. Jiang, W.-S. Kim, Recent progress on magnetic iron oxide nanoparticles: synthesis, surface functional strategies and biomedical applications, *Sci. Technol. Adv. Mater.* 16 (2015) 023501. doi:10.1088/1468-6996/16/2/023501.
- [10] M. Ahmadzadeh, C. Romero, J. McCloy, Magnetic analysis of commercial hematite, magnetite, and their mixtures, *AIP Adv.* 8 (2018) 056807. doi:10.1063/1.5006474.
- [11] R. Dronskowski, The Little Maghemite Story: A Classic Functional Material, *Adv. Funct. Mater.* 11 (2001) 27–29. doi:10.1002/1616-3028(200102)11:1<27::AID-ADFM27>3.0.CO;2-X.
- [12] A.S. Teja, P.-Y. Koh, Synthesis, properties, and applications of magnetic iron oxide nanoparticles, *Prog. Cryst. Growth Charact. Mater.* 55 (2009) 22–45. doi:10.1016/j.pcrysgrow.2008.08.003.
- [13] S. Bedanta, W. Kleemann, Supermagnetism, *J. Phys. D. Appl. Phys.* 42 (2009) 013001. doi:10.1088/0022-3727/42/1/013001.
- [14] T. Macher, J. Totenhagen, J. Sherwood, Y. Qin, D. Gurler, M.S. Bolding, Y. Bao, Ultrathin Iron Oxide Nanowhiskers as Positive Contrast Agents for Magnetic Resonance Imaging, *Adv. Funct. Mater.* 25 (2015) 490–494. doi:10.1002/adfm.201403436.
- [15] L.M. Rossi, N.J.S. Costa, F.P. Silva, R. Wojcieszak, Magnetic nanomaterials in catalysis: advanced catalysts for magnetic separation and beyond, *Green Chem.* 16

(2014) 2906. doi:10.1039/c4gc00164h.

- [16] J. Liu, Z. Wu, Q. Tian, W. Wu, X. Xiao, Shape-controlled iron oxide nanocrystals: synthesis, magnetic properties and energy conversion applications, *CrystEngComm*. 18 (2016) 6303–6326. doi:10.1039/C6CE01307D.
- [17] L.-L. Tian, M.-J. Zhang, C. Wu, Y. Wei, J.-X. Zheng, L.-P. Lin, J. Lu, K. Amine, Q.-C. Zhuang, F. Pan, γ -Fe₂O₃ Nanocrystalline Microspheres with Hybrid Behavior of Battery-Supercapacitor for Superior Lithium Storage, *ACS Appl. Mater. Interfaces*. 7 (2015) 26284–26290. doi:10.1021/acsami.5b08756.
- [18] A.M. Bruck, C.A. Cama, C.N. Gannett, A.C. Marschilok, E.S. Takeuchi, K.J. Takeuchi, Nanocrystalline iron oxide based electroactive materials in lithium ion batteries: the critical role of crystallite size, morphology, and electrode heterostructure on battery relevant electrochemistry, *Inorg. Chem. Front.* 3 (2016) 26–40. doi:10.1039/C5QI00247H.
- [19] S. Sun, C.B. Murray, D. Weller, L. Folks, A. Moser, Monodisperse FePt Nanoparticles and Ferromagnetic FePt Nanocrystal Superlattices, *Science* (80-.). 287 (2000) 1989–1992. doi:10.1126/science.287.5460.1989.
- [20] G. Liu, J. Gao, H. Ai, X. Chen, Applications and Potential Toxicity of Magnetic Iron Oxide Nanoparticles, *Small*. 9 (2013) 1533–1545. doi:10.1002/smll.201201531.
- [21] P. Xu, G.M. Zeng, D.L. Huang, C.L. Feng, S. Hu, M.H. Zhao, C. Lai, Z. Wei, C. Huang, G.X. Xie, Z.F. Liu, Use of iron oxide nanomaterials in wastewater treatment: A review, *Sci. Total Environ.* 424 (2012) 1–10. doi:10.1016/j.scitotenv.2012.02.023.
- [22] P. Fraga García, M. Brammen, M. Wolf, S. Reinlein, M. Freiherr von Roman, S. Berensmeier, High-gradient magnetic separation for technical scale protein recovery using low cost magnetic nanoparticles, *Sep. Purif. Technol.* 150 (2015) 29–36. doi:10.1016/j.seppur.2015.06.024.
- [23] M. Franzreb, M. Siemann-Herzberg, T.J. Hobley, O.R.T. Thomas, Protein purification using magnetic adsorbent particles, *Appl. Microbiol. Biotechnol.* 70 (2006) 505–516. doi:10.1007/s00253-006-0344-3.
- [24] A. Jordan, P. Wust, H. Fählin, W. John, A. Hinz, R. Felix, Inductive heating of ferrimagnetic particles and magnetic fluids: Physical evaluation of their potential for hyperthermia, *Int. J. Hyperth.* 9 (1993) 51–68. doi:10.3109/02656739309061478.
- [25] G. Kandasamy, A. Sudame, T. Luthra, K. Saini, D. Maity, Functionalized Hydrophilic Superparamagnetic Iron Oxide Nanoparticles for Magnetic Fluid Hyperthermia Application in Liver Cancer Treatment, *ACS Omega*. 3 (2018) 3991–4005. doi:10.1021/acsomega.8b00207.
- [26] T. Kobayashi, Cancer hyperthermia using magnetic nanoparticles, *Biotechnol. J.* 6 (2011) 1342–1347. doi:10.1002/biot.201100045.
- [27] M. Barrow, A. Taylor, D.J. Nieves, L.K. Bogart, P. Mandal, C.M. Collins, L.R. Moore, J.J. Chalmers, R. Lévy, S.R. Williams, P. Murray, M.J. Rosseinsky, D.J. Adams, Tailoring the surface charge of dextran-based polymer coated SPIONs for modulated stem cell uptake and MRI contrast, *Biomater. Sci.* 3 (2015) 608–616. doi:10.1039/C5BM00011D.
- [28] M. Bañobre-López, A. Teijeiro, J. Rivas, Magnetic nanoparticle-based hyperthermia

for cancer treatment, *Reports Pract. Oncol. Radiother.* 18 (2013) 397–400. doi:10.1016/j.rpor.2013.09.011.

- [29] M.R. Reagan, D.L. Kaplan, Concise Review: Mesenchymal Stem Cell Tumor-Homing: Detection Methods in Disease Model Systems, *Stem Cells*. 29 (2011) 920–927. doi:10.1002/stem.645.
- [30] U. Altanerova, M. Babincova, P. Babinec, K. Benejova, J. Jakubechova, V. Altanerova, M. Zduriencikova, V. Repiska, C. Altaner, Human mesenchymal stem cell-derived iron oxide exosomes allow targeted ablation of tumor cells via magnetic hyperthermia, *Int. J. Nanomedicine*. Volume 12 (2017) 7923–7936. doi:10.2147/IJN.S145096.
- [31] P.K. Sneed, P.R. Stauffer, M.W. McDermott, C.J. Diederich, K.R. Lamborn, M.D. Prados, S. Chang, K.A. Weaver, L. Spry, M.K. Malec, S.A. Lamb, B. Voss, R.L. Davis, W.M. Wara, D.A. Larson, T.L. Phillips, P.H. Gutin, Survival benefit of hyperthermia in a prospective randomized trial of brachytherapy boost $\pm$ hyperthermia for glioblastoma multiforme, *Int. J. Radiat. Oncol.* 40 (1998) 287–295. doi:10.1016/S0360-3016(97)00731-1.
- [32] S. Kaushik, J. Thomas, V. Panwar, H. Ali, V. Chopra, A. Sharma, R. Tomar, D. Ghosh, In Situ Biosynthesized Superparamagnetic Iron Oxide Nanoparticles (SPIONS) Induce Efficient Hyperthermia in Cancer Cells, *ACS Appl. Bio Mater.* 3 (2020) 779–788. doi:10.1021/acsabm.9b00720.
- [33] J. Dulińska-Litewka, A. Łazarczyk, P. Hałubiec, O. Szafranski, K. Karnas, A. Karewicz, Superparamagnetic Iron Oxide Nanoparticles—Current and Prospective Medical Applications, *Materials (Basel)*. 12 (2019) 617. doi:10.3390/ma12040617.
- [34] C. Alexiou, R.J. Schmid, R. Jurgons, M. Kremer, G. Wanner, C. Bergemann, E. Huenges, T. Nawroth, W. Arnold, F.G. Parak, Targeting cancer cells: magnetic nanoparticles as drug carriers, *Eur. Biophys. J.* 35 (2006) 446–450. doi:10.1007/s00249-006-0042-1.
- [35] N. Kohler, C. Sun, A. Fichtenholtz, J. Gunn, C. Fang, M. Zhang, Methotrexate-Immobilized Poly(ethylene glycol) Magnetic Nanoparticles for MR Imaging and Drug Delivery, *Small*. 2 (2006) 785–792. doi:10.1002/sml.200600009.
- [36] K. Cheng, S. Peng, C. Xu, S. Sun, Porous Hollow Fe₃O₄ Nanoparticles for Targeted Delivery and Controlled Release of Cisplatin, *J. Am. Chem. Soc.* 131 (2009) 10637–10644. doi:10.1021/ja903300f.
- [37] C. Yang, A. Rait, K.F. Pirollo, J.A. Dagata, N. Farkas, E.H. Chang, Nanoimmunoliposome delivery of superparamagnetic iron oxide markedly enhances targeting and uptake in human cancer cells in vitro and in vivo, *Nanomedicine Nanotechnology, Biol. Med.* 4 (2008) 318–329. doi:10.1016/j.nano.2008.05.004.
- [38] Y. Bao, J.A. Sherwood, Z. Sun, Magnetic iron oxide nanoparticles as T1 contrast agents for magnetic resonance imaging, *J. Mater. Chem. C*. 6 (2018) 1280–1290. doi:10.1039/C7TC05854C.
- [39] M. Legacz, K. Roepke, M. Giersig, U. Pison, Contrast Agents and Cell Labeling Strategies for in Vivo Imaging, *Adv. Nanoparticles*. 03 (2014) 41–53. doi:10.4236/anp.2014.32007.
- [40] L. Li, W. Jiang, K. Luo, H. Song, F. Lan, Y. Wu, Z. Gu, Superparamagnetic Iron Oxide Nanoparticles as MRI contrast agents for Non-invasive Stem Cell Labeling and

Tracking, Theranostics. 3 (2013) 595–615. doi:10.7150/thno.5366.

- [41] R. Qiao, C. Yang, M. Gao, Superparamagnetic iron oxide nanoparticles: from preparations to in vivo MRI applications, *J. Mater. Chem.* 19 (2009) 6274. doi:10.1039/b902394a.
- [42] A. Taylor, K.M. Wilson, P. Murray, D.G. Fernig, R. Lévy, Long-term tracking of cells using inorganic nanoparticles as contrast agents: are we there yet?, *Chem. Soc. Rev.* 41 (2012) 2707. doi:10.1039/c2cs35031a.
- [43] J. Sherwood, M. Rich, K. Lovas, J. Warram, M.S. Bolding, Y. Bao, T1 -Enhanced MRI-visible nanoclusters for imaging-guided drug delivery, *Nanoscale.* 9 (2017) 11785–11792. doi:10.1039/C7NR04181K.
- [44] J.S. Guthi, S.-G. Yang, G. Huang, S. Li, C. Khemtong, C.W. Kessinger, M. Peyton, J.D. Minna, K.C. Brown, J. Gao, MRI-Visible Micellar Nanomedicine for Targeted Drug Delivery to Lung Cancer Cells, *Mol. Pharm.* 7 (2010) 32–40. doi:10.1021/mp9001393.
- [45] L. Ren, S. Chen, H. Li, Z. Zhang, J. Zhong, M. Liu, X. Zhou, MRI-guided liposomes for targeted tandem chemotherapy and therapeutic response prediction, *Acta Biomater.* 35 (2016) 260–268. doi:10.1016/j.actbio.2016.02.011.
- [46] Z. Jin, Y. Wen, Y. Hu, W. Chen, X. Zheng, W. Guo, T. Wang, Z. Qian, B.-L. Su, Q. He, MRI-guided and ultrasound-triggered release of NO by advanced nanomedicine, *Nanoscale.* 9 (2017) 3637–3645. doi:10.1039/C7NR00231A.
- [47] A.S. Arbab, J.A. Frank, Cellular MRI and its role in stem cell therapy, *Regen. Med.* 3 (2008) 199–215. doi:10.2217/17460751.3.2.199.
- [48] G. Fleige, C. Nolte, M. Synowitz, F. Seeberger, H. Kettenmann, C. Zimmer, Magnetic Labeling of Activated Microglia in Experimental Gliomas, *Neoplasia.* 3 (2001) 489–499. doi:10.1038/sj.neo.7900176.
- [49] H. Xing, S. Zhang, W. Bu, X. Zheng, L. Wang, Q. Xiao, D. Ni, J. Zhang, L. Zhou, W. Peng, K. Zhao, Y. Hua, J. Shi, Ultrasmall NaGdF₄ Nanodots for Efficient MR Angiography and Atherosclerotic Plaque Imaging, *Adv. Mater.* 26 (2014) 3867–3872. doi:10.1002/adma.201305222.
- [50] D. Ni, Z. Shen, J. Zhang, C. Zhang, R. Wu, J. Liu, M. Yi, J. Wang, Z. Yao, W. Bu, J. Shi, Integrating Anatomic and Functional Dual-Mode Magnetic Resonance Imaging: Design and Applicability of a Bifunctional Contrast Agent, *ACS Nano.* 10 (2016) 3783–3790. doi:10.1021/acsnano.6b00462.
- [51] M. Rudin, M. Rausch, M. Stoeckli, Molecular Imaging in Drug Discovery and Development: Potential and Limitations of Nonnuclear Methods, *Mol. Imaging Biol.* 7 (2005) 5–13. doi:10.1007/s11307-004-0954-z.
- [52] C. Shao, F. Liu, W. Le, T. Mei, T. Wang, L. Chen, Y. Lei, S. Cui, B. Chen, Z. Cui, In vitro and in vivo targeting imaging of pancreatic cancer using a Fe₃O₄@SiO₂ nanoprobe modified with anti-mesothelin antibody, *Int. J. Nanomedicine.* (2016) 2195. doi:10.2147/IJN.S104501.
- [53] N.G. Ordóñez, Application of Mesothelin Immunostaining in Tumor Diagnosis, *Am. J. Surg. Pathol.* 27 (2003) 1418–1428. doi:10.1097/00000478-200311000-00003.
- [54] S. Boutry, S. Laurent, L. Vander Elst, R.N. Muller, Specific E-selectin targeting with a

superparamagnetic MRI contrast agent, *Contrast Media Mol. Imaging.* 1 (2006) 15–22. doi:10.1002/cmmi.87.

- [55] Y. Okuhata, Delivery of diagnostic agents for magnetic resonance imaging, *Adv. Drug Deliv. Rev.* 37 (1999) 121–137. doi:10.1016/S0169-409X(98)00103-3.
- [56] A. Szpak, S. Fiejdasz, W. Prendota, T. Strączek, C. Kapusta, J. Szmyd, M. Nowakowska, S. Zapotoczny, T1–T2 Dual-modal MRI contrast agents based on superparamagnetic iron oxide nanoparticles with surface attached gadolinium complexes, *J. Nanoparticle Res.* 16 (2014) 2678. doi:10.1007/s11051-014-2678-6.
- [57] Z. Zhou, R. Bai, J. Munasinghe, Z. Shen, L. Nie, X. Chen, T1 – T2 Dual-Modal Magnetic Resonance Imaging: From Molecular Basis to Contrast Agents, *ACS Nano.* 11 (2017) 5227–5232. doi:10.1021/acsnano.7b03075.
- [58] V.K. Sharma, A. Alipour, Z. Soran-Erdem, Z.G. Aykut, H. V. Demir, Highly monodisperse low-magnetization magnetite nanocubes as simultaneous T1 – T2 MRI contrast agents, *Nanoscale.* 7 (2015) 10519–10526. doi:10.1039/C5NR00752F.
- [59] Z. Shen, A. Wu, X. Chen, Iron Oxide Nanoparticle Based Contrast Agents for Magnetic Resonance Imaging, *Mol. Pharm.* 14 (2017) 1352–1364. doi:10.1021/acs.molpharmaceut.6b00839.
- [60] G. Huang, H. Li, J. Chen, Z. Zhao, L. Yang, X. Chi, Z. Chen, X. Wang, J. Gao, Tunable T1 and T2 contrast abilities of manganese-engineered iron oxide nanoparticles through size control, *Nanoscale.* 6 (2014) 10404. doi:10.1039/C4NR02680B.
- [61] F. Hu, Q. Jia, Y. Li, M. Gao, Facile synthesis of ultrasmall PEGylated iron oxide nanoparticles for dual-contrast T1 - and T2 -weighted magnetic resonance imaging, *Nanotechnology.* 22 (2011) 245604. doi:10.1088/0957-4484/22/24/245604.
- [62] Z. Li, P.W. Yi, Q. Sun, H. Lei, H. Li Zhao, Z.H. Zhu, S.C. Smith, M.B. Lan, G.Q.M. Lu, Ultrasmall Water-Soluble and Biocompatible Magnetic Iron Oxide Nanoparticles as Positive and Negative Dual Contrast Agents, *Adv. Funct. Mater.* 22 (2012) 2387–2393. doi:10.1002/adfm.201103123.

CHAPTER 8

Simple co-precipitation synthesis of USPION suitable as T1 MRI contrast agents

As mentioned previously, Contrast Agents (CA) are commonly used in MRI to speed up either the longitudinal (T1) or transverse (T2) relaxation process, enhancing the local contrast in pathological tissue to produce more detailed images. Normally, CA act on both longitudinal and transverse relaxation, although one is usually dominant and it determines the type of CA: if a species primarily affects T1 relaxation, it is referred as T1-type, if instead it mainly influences the T2 process, it is called T2-type. The first produces positive contrast, brightening the affected region, while the latter provides negative contrast, which darkens it.

T1 relaxation times depend on the spin energy dissipation to the surrounding environment, so direct coordination of water molecules to the CA and water exchange rates are crucial. For clinical applications, the main choice usually falls on gadolinium complexes, T1 contrast agents that provide positive contrast, favoured for better resolution and detection of anomalies [1]. On the other hand, Gd complexes present a serious health risk for those patients unable to efficiently remove these heavy metal complexes due to pre-existent kidney or liver pathologies[2]. Moreover, Gd-based CA have been reported to cause undesirable accumulation in the brain and consequential tissue damage even in healthy patients[3,4]. To address these issues, modern Gd-based CA are designed to have short body retention times, although this limits their application in therapy monitoring[5].

In the case of transverse relaxation, T2 times depend on how fast the spin moments dephase, shifting the attention on the magnetic properties of the CA. T2 CA are normally represented by superparamagnetic iron oxide nanoparticles, which have proven to be one of the most promising CA for clinical use, as they are naturally found in the human body and can be detected in smaller concentrations[6]. Nonetheless, they are less favoured by radiologists because the darker tones they provide can be mistaken with low resolution and background interference caused by body fluids, voids and endogenous metal deposits[1,7]. Even though, in the last few years, a series of attempts have been made to convert T2 CA-generated contrast from negative to positive[8], Gd-based CA still represent the first choice of radiologists around the world.

While most SPION act as T2 CA, several papers reported their potential use as T1 CA, given their size is sufficiently small, indicatively less than 4 nm[9,10]. These materials are known as Ultrasmall SPION, or USPION, and their diffusion and study in the medical field has seen a noticeable increase over the last ten years. Although the exact mechanisms behind the T1 enhancement of these materials still require further investigation, their large surface area is at the basis of their functioning, increasing the interface between metal ions and water molecules[10]. The need for a safer alternative

to Gd-based contrast agents has raised interest towards the development of biocompatible nanosized T1 contrast agents and the long-lasting biocompatibility of iron oxide makes USPION highly promising candidates for T1 image enhancement, as they could provide both efficiency and safety.

Ultrasmall iron oxide nanoparticles can be synthesised through a variety of procedures, which can be divided in three main categories: iron salts reduction, thermal degradation and co-precipitation. The first two are usually performed in organic solvents, while the last one is typically a water-based synthetic route.

Iron salts reduction is carried out in the presence of polyols such as ethylene glycol or polyethylene glycol, which act as both reducing and stabilizing agents, at temperatures ranging from 150°C to 300°C[11]. The use of microwaves and stronger reducing agents such as hydrazine have also been reported[12]. While one-pot syntheses based on polyols have gathered attention thanks to their simplicity and ease of scale-up, the colloidal stability of the resulting USPION represents a major issue, as particles prepared by these procedures tend to aggregate[10].

High temperature thermal decomposition is widely adopted in the literature to produce high quality iron oxide nanoparticles with great reproducibility and narrow size distribution. This approach exploits the thermal decomposition of iron precursors, such as iron oleate or iron acetylacetonate, in the presence of suitable stabilizers. To control particle growth, temperatures are usually kept at lower values, around 260 °C, compared to those adopted for larger particles which can reach over 300 °C. Furthermore, strong growth inhibitors, such as hexadecanediol, are added mid synthesis and the reaction is quenched shortly after the nucleation phase: this way, USPION as small as 3 nm can be obtained[13]. Despite the superior control over particle crystallinity and morphology, the synthetic requirements of this technique are relatively demanding, as reactions must be carried out at high temperature, by carefully supervising heating rates, and under an inert atmosphere. Moreover, the resulting particles are covered in organic long-chained molecules like oleate, making them non-water-dispersible if not further subjected to ligand exchange procedures[14].

Lastly, co-precipitation involves the use of ferric and ferrous salts as precursors, mixed in a 2:1 ratio, hydrolysed through the use of a base, such as NaOH or NH₄OH, to produce iron oxide nanoparticles. It generally produces larger and more polydispersed nanoparticles compared to the previous synthetic routes, but it can be modified to prepare smaller, monodispersed samples. High-temperature co-precipitation was proved to produce USPION as small as 3 nm, but it required some modifications and careful operational precision, such as the use of hot aqueous polymer solutions[6] or the addition of the base on a milliseconds timescale, under rigorously controlled temperatures[15]. Compared to the previous methods, the co-precipitation approach has the lowest synthetic requirements, as it can be performed at room temperature using low-cost chemicals with the least amount of time. Furthermore, produced particles are generally very stable and come already dispersed in water, which is fundamental for biological applications.

The aim of this work was to develop a simple co-precipitation synthetic protocol to prepare USPION suitable as MRI contrast agents. The disadvantage of co-precipitation syntheses lies in the inherently lower control over crystallinity and morphology of the

resulting nanoparticles, whereas thermolysis allows for far more precise particle design. Normally, nanoparticles prepared via co-precipitation show larger size, broader size distribution and lower crystallinity, due to the critical role played by local currents and concentrations, which are difficult to control. To define the optimal synthetic protocol, several different parameters were changed to investigate their role in the preparation of iron oxide nanoparticles. These comprised reaction temperature, base type, purification procedure, stabilizing molecules and precursor concentration. In this way, through a combination of the right factors, it was possible to achieve a significantly small and narrow USPION population, which remained stable for months thanks to the peptizing effect of citrate ions adsorbed on the surface.

8.1 Experimental procedures

8.1.1 Materials

Chemicals, of analytical grade, were acquired from Sigma-Aldrich (unless differently stated) and were used without further purification. Water was doubly distilled and deionised through a Milli-Q apparatus.

8.1.2 Synthesis of SPION

The synthesis of SPION was carried out according to the following general procedure. Precursor aqueous solutions, either 0.5 M or 1 M, were prepared by dissolving the necessary amount of Fe salts in Milli-Q water. 2.5 mL of the $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution was mixed with 1.25 mL of the $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution in a beaker. Syntheses were performed either at room temperature or at 50°C in a water bath. Then, in selected syntheses, a stabilizing agent (1-3% w/w sodium oleate or 1 mL of 1.6 M citric acid solution) was added (for more details refer to Sections 8.1.3.1 and 8.1.3.3). The reaction mixture was magnetically stirred for 10 minutes (500 rpm). The pH was raised by dropwise addition of 6.25 mL of the base solution, either NH_4OH 25% w/w, tetramethylammonium hydroxide (TMAOH) 25% w/w or NaOH 3 M, and the solution, which immediately assumed a dark colour, was kept stirring for 20 minutes with a magnetic stirrer (1000 rpm). The reaction led to a stable black suspension. Table 8.1 lists the prepared samples with their acronyms and main synthetic parameters.

Sample	Base	Temperature (°C)	Stabilizer	Precursor concentration (mol L ⁻¹)
USPt	TMAOH	25	-	1
USPa	NH ₄ OH	25	-	1
USPs	NaOH	25	-	1
USPt50	TMAOH	50	-	1
USPa50	NH ₄ OH	50	-	1
USPs50	NaOH	50	-	1
USPa-SO1	NH ₄ OH	25	Sodium oleate 1%	1
USPa-SO3	NH ₄ OH	25	Sodium oleate 3%	1
USPt-APTES	TMAOH	25	APTES	1
USPt-CApost	TMAOH	25	Citric acid after growth	1
USPt-CApre	TMAOH	25	Citric acid before growth	1
USPt-CApre05	TMAOH	25	Citric acid before growth	0.5

Table 8.1 - List of prepared samples with the relative synthetic details.

Moreover, sample names present a suffix depending on the adopted purification procedure. Dialysed samples exhibit a suffix depending on the liquid adopted for the procedure: _dW for water, _dCA for citric acid 0.08 M and _dSC for sodium citrate 0.08 M. in the same way, centrifuged samples show a suffix indicating the relative centrifugal force (RCF) adopted: _c18 for 18000 and _c300 for 360000 RCF.

8.1.3 Functionalization with different stabilizing agents

8.1.3.1 Sodium oleate

Sodium oleate was added to Fe precursor solutions (1 M) to achieve a weight fraction of either 1% or 3%. These were mixed and stirred, as explained in the general procedure. To raise the pH, a previously prepared basic solution (NH₄OH 25% w/w, sodium oleate 1% or 3%) was used. The solution was kept stirring for 20 minutes with a magnetic stirrer (1000 rpm).

8.1.3.2 APTES

A fraction of USPION prepared with TMAOH were functionalised following this procedure: 1 mL of USPION suspension was added to 23 mL of 2-propanol and stirred magnetically (1000 rpm). Right after, 0.5 mL (2.13 mmol) of APTES was added. The suspension was heated at 70°C in an oil bath and stirred for 1 h. At the end of the reaction, the suspension was left cooling down at RT. It was then centrifuged and re-dispersed in 2-propanol 4 times. After the last washing cycle, nanoparticles were dispersed in water.

8.1.3.3 Citric acid

Citric acid addition was tested before and after particle precipitation: 1 mL of a citric acid solution (1.6 M) was added to the initial mixture of Fe precursor solutions. In a second set of experiments, the same amount of 1.6 M citric acid was added at once to the reaction mixture after 20 min and then magnetically stirred for 1 h.

8.1.4 Purification procedures

Several purification methods were tested at the end of the general synthetic procedure. The purification was made challenging by the small size of the particles, which in most cases precluded the separation of the suspended solid, and by the need to retain the adsorbed stabilizing agent to preserve the chemical stability and redispersibility of the particles.

8.1.4.1 Magnetic decantation

A Nd magnet was put underneath the reactor until a solid residue was settled. This process could take a variable amount of time, depending on the relative colloidal stability of the suspension, ranging from 20 minutes to 1 h. After sedimentation, the supernatant was gently poured out and substituted with fresh solvent. The procedure was repeated up to 3 times.

8.1.4.2 Centrifugation

Different centrifugation protocols were tested: 18000 RCF for 1 h; 360000 RCF for 20 minutes and 20 h.

8.1.4.3 Dialysis and centrifuge-aided dialysis

Traditional dialysis was performed using cellulose membranes (Sigma-Aldrich, cut-off 14 kD or 3 kD). Before usage, the membrane was rinsed with running tap water, then washed in a 60 °C Milli-Q water bath for few minutes. After loading with the SPION suspension (10 mL), the membrane was immersed in 550 mL of the chosen liquid, either Milli-Q water, a 0.08 M solution of citric acid or a 0.08 M solution of tribasic sodium citrate. It was left resting for 24 h before retrieving the purified suspension.

Centrifuge-aided dialysis was performed by pouring 0.5 mL of USPION suspension inside centrifuge tubes equipped with a cellulose filter (Amicon, cut-off 3 kD) and adding 5 mL of Milli-Q water. Tubes were centrifuged at 7200 RCF for 10 minutes, repeating the procedure 4 times.

8.1.5 Sample characterizations

Average particle size determinations were performed on SPION suspensions diluted in Milli-Q water by Dynamic Light Scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments), operating at $\lambda = 633$ nm with a solid state He-Ne laser at a scattering angle of 173°. Each hydrodynamic diameter was averaged from at least three measurements.

The isoelectric point (pH_{IEP}) was evaluated determining the oxide ζ -potential on a range of pH values with the same instrument, using disposable dip-cells. For ζ -potential measurements, suspensions were diluted with a 0.01 M KNO_3 aqueous solution, while pH was adjusted with 0.01 M KOH and HNO_3 solutions.

Transmission electron microscopy (TEM) images were acquired using a Jeol JEM-1400 plus TEM, operating at an acceleration voltage of 120 kV. Samples were dispersed in either 2-propanol (0.5 mg mL^{-1}) and deposited on Cu holey carbon grids (200 mesh). Particle size was evaluated through the use of image analysis software ImageJ, measuring 320 individual particles per sample.

Fourier Transform Infrared (FTIR) spectra were registered using two separate instruments, due to the access limitations:

- A Thermofischer Nicolet 6700 spectrophotometer working in Attenuated Total Reflectance (ATR) mode, acquiring 128 scans between 4000 and 650 cm^{-1} with a resolution of 4.0 cm^{-1} .
- A PerkinElmer Frontier spectrophotometer in ATR mode, acquiring 24 scans between 4000 and 400 cm^{-1} with a resolution of 4.0 cm^{-1} .

For FTIR spectra measurements, a certain amount of USPION suspension was dried on a hot plate and deposited on the Zn/Se crystal of the instrument for analysis.

8.2 Results and discussion

8.2.1 Effect of reaction temperature

As the co-precipitation synthesis of nanoparticles is based on a delicate equilibrium between nucleation and growth rate, temperature can be of critical importance for tuning the morphology. For this reason, a series of tests were performed to assess the effects of a temperature change in the reaction medium. Syntheses were carried out at room temperature and at 50°C and were repeated for each base under study (NH_4OH , TMAOH and NaOH): the goal was to verify whether raising the temperature could increase nucleation rates and hinder particle growth, as previously reported in the literature[16], while also gauging the effect of temperature on colloidal stability.

Raising the reaction temperature to 50°C had a different effect depending on the type of base used. The most noticeable effect was found for samples synthesised using NaOH, namely USPs and USPs50: in this case, increasing the temperature caused the degradation of nanoparticles, which turned brown and did not respond to the field of a strong Nd magnet. As shown in Figure 8.1A and Figure 8.1B, in the case of samples prepared with NH_4OH (USPa and USPa50), the median size increased significantly, from $6.2 \pm 1.3 \text{ nm}$ to $9.5 \pm 2.0 \text{ nm}$. In the case of TMAOH-prepared samples (USPt and USPt50), reported in Figure 8.1C and Figure 8.1D, median particle size remain comparable within experimental error, $7.3 \pm 1.3 \text{ nm}$ and $7.0 \pm 1.4 \text{ nm}$ for USPt and USPt50, respectively. On the other hand, colloidal stability moderately worsened, as evidenced by the DLS curves in Figure 8.2.

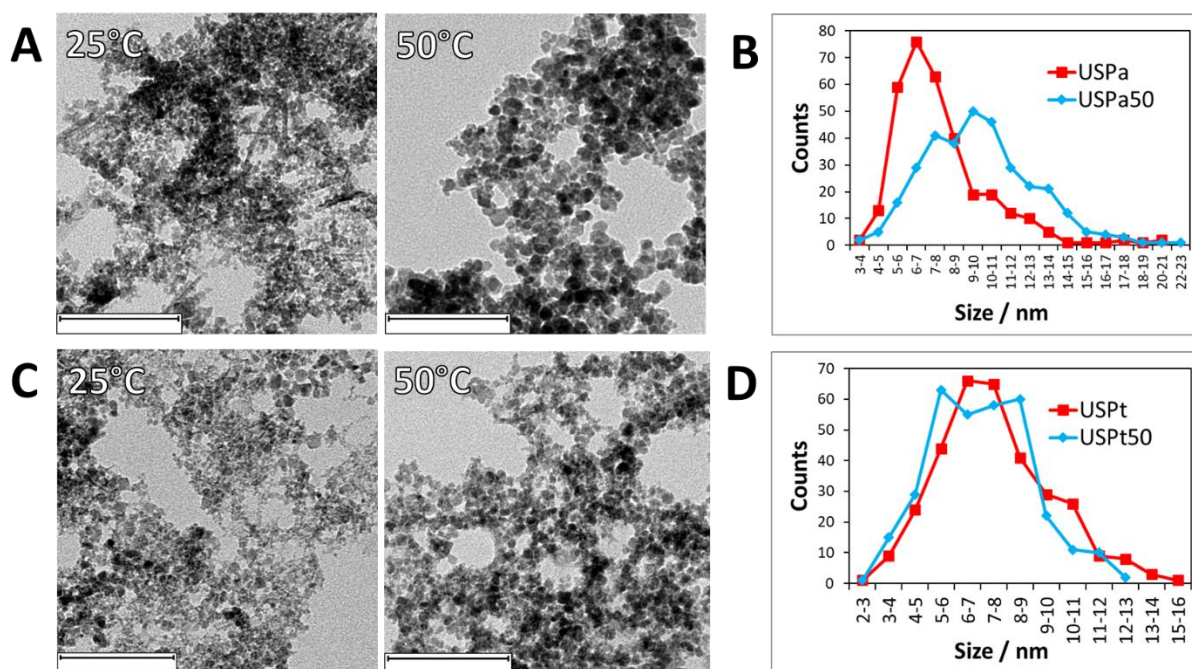


Figure 8.1 - TEM images and particle size distribution of samples prepared at 25°C and 50°C with NH_4OH (A, B) and TMAOH (C, D). Scale bar is 100 nm.

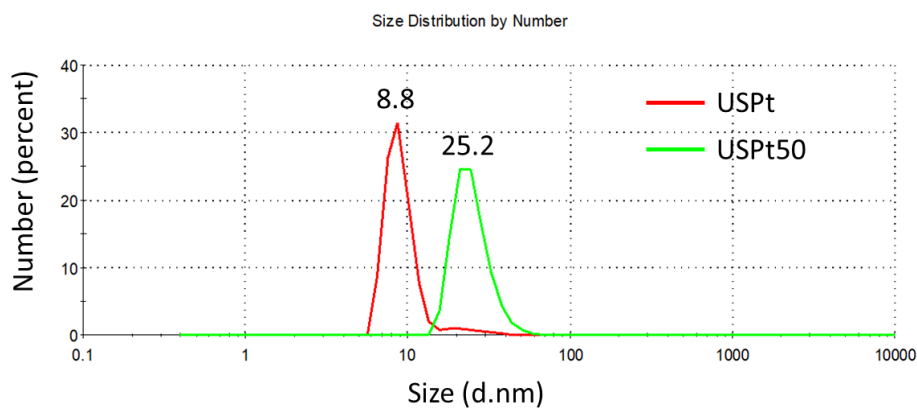


Figure 8.2 - Hydrodynamic diameter measured by DLS for samples USPt and USPt50.

In light of these results, it can be assumed that raising reaction temperature has little to no benefit for the preparation of USPION using the present synthetic procedure, leading to either size increase or even particle degradation. For this reason, all other experiments were performed at RT.

8.2.2 Effect of base type

The nature of the alkaline agent plays a crucial role in determining the interfacial properties of magnetic nanoparticles, as the cation usually adsorbs, either physically or

chemically, on the oxide surface during nucleation and growth. This phenomenon can greatly affect surface charge and steric hindrance, thus influencing the colloidal stability of the suspension[17]. With the aim of finding the ideal base type, multiple synthetic tests were performed with three different alkaline molecules: ammonium hydroxide (NH_4OH), tetramethylammonium hydroxide ($\text{N}(\text{CH}_3)_4\text{OH}$, TMAOH) and sodium hydroxide (NaOH).

Initially, the effect of NH_4OH was compared to that of TMAOH: the latter is a better peptizing agent[17] and a stronger base, but it is also far more toxic than ammonia solution, so the aim was to assess whether the benefits of TMAOH would outweigh its drawbacks. When looking at single particle size from TEM images, the two bases behave very similarly, producing particles with a median size of *ca.* 7 nm, as previously shown in Figure 8.1. The main advantage of TMAOH becomes apparent when comparing DLS data. DLS measurements give information on the hydrodynamic size of particles in solution, which, if the actual particle size is known, provides a means of assessing the respective colloidal stability and aggregation degree. In Figure 8.3, the size distribution curves, expressed by particles number, highlight a significant difference between the two reported samples, as the hydrodynamic diameter of USP_a seems to be distributed into two populations, one centred at 140 nm and the other at around 350 nm, while that of USP_t is narrowly centred at 9 nm. Approximating using the Random Close Packing parameter for hard spheres, we can elaborate these data to estimate the number of aggregated particles: it has been defined that, for randomly packed spheroids, the maximum packing efficiency η is around 0.64, which is significantly lower than the optimal packing density for cubic or hexagonal close packing of 0.74[18]. With this parameter, given the particle size median (d), obtained from TEM image analysis, and average hydrodynamic diameter (D), we can calculate the number of aggregated particles N_{agg} with the following equation:

$$N_{agg} = \frac{\frac{4}{3}\pi\left(\frac{D}{2}\right)^3 \times 0.64}{\frac{4}{3}\pi\left(\frac{d}{2}\right)^3}$$

Which, in the case of particles synthesised with ammonia, suggests aggregates of up to 100,000 individuals. On the other hand, those prepared with TMAOH for the most part are separated from each other, as their low hydrodynamic diameter suggests. As tetramethylammonium hydroxide is a stronger base than ammonia ($\text{pK}_b < 2$ vs. 4.7), it was hypothesised that their dissociation constants could have an effect on their peptizing capabilities. For this reason, NaOH was chosen as a control alkaline agent, as its strength is comparable to TMAOH, but it leaves much fewer toxic residues in the product.

The TEM images reported in Figure 8.4 show glaring differences between the two samples: the first thing that catches the attention is the presence of large nanorods alongside spherical particles in sample USP_s, prepared using NaOH . Moreover, NaOH -prepared USPION appears to be moderately larger than particles synthesised with TMAOH, having a median of 8.3 ± 1.7 nm. Comparing their hydrodynamic diameter, the presence of the abovementioned nanorods, together with the slightly bigger

particles, translate to larger aggregates, which, even if smaller than those produced by NH_4OH , still measure around 50 nm, as reported in Figure 8.3.

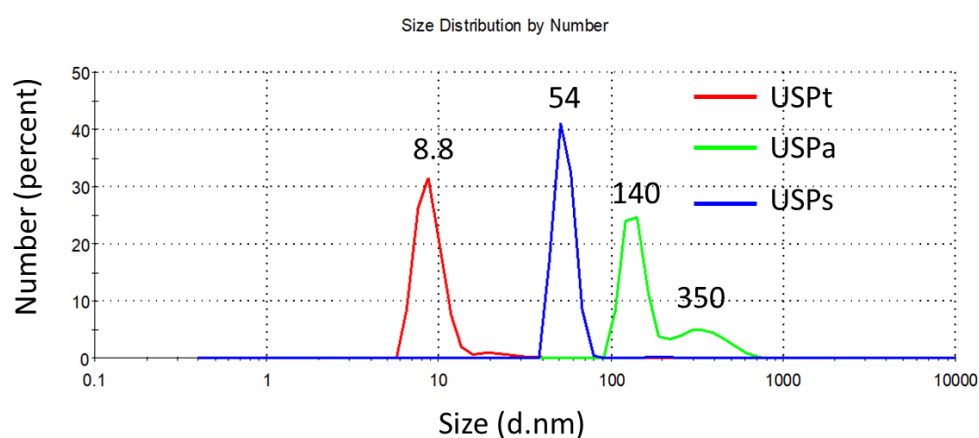


Figure 8.3 - Hydrodynamic diameter measured by DLS for USPION prepared with TMAOH, NH_4OH and NaOH .

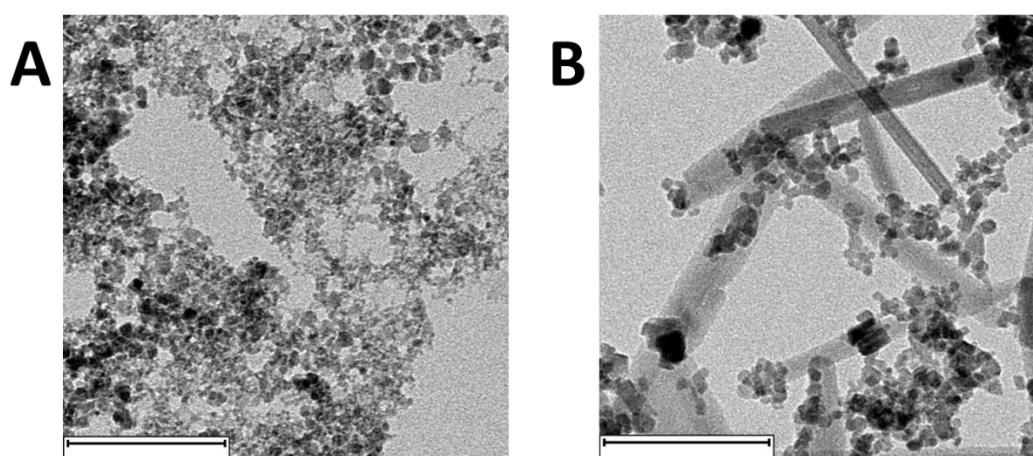


Figure 8.4 - TEM images of sample USPt (A) and USPs (B). Scale bar is 100 nm.

These results suggest that the peptizing effect of TMAOH is extremely significant and valuable, as colloidal stability and resistance to particle aggregation are paramount to guarantee the correct behaviour of diagnostic nanosystems, reducing the risk of clots formation and blood vessel obstruction.

8.2.3 Effect of stabilizing agents

The colloidal stability of a suspension most often depends on the presence of stabilizing agents, which can act either on the steric hindrance between particles or on their surface charge to prevent them from aggregating and settling. When designing nanometric biomaterials, colloidal stability is paramount, especially when dealing with treatments administered intravenously: particle aggregation could lead to the obstruction of blood vessels and the formation of potentially lethal blood clots. Furthermore, these stabilizing molecules must also possess high biocompatibility, as the introduction of cytotoxic or otherwise hazardous substances in the organism could aggravate the patient's health condition.

One of the tested parameters during the optimization of the synthetic procedure was indeed the use of biocompatible stabilizing agents to improve the colloidal stability of prepared USPION suspensions. Three specific compounds were chosen for these tests: sodium oleate, (3-aminopropyl)triethoxy silane (APTES) and citric acid. All these three molecules are reportedly biocompatible and are commonly adopted in the literature to functionalize the surface of many different nanosystems[19–21].

8.2.3.1 Sodium oleate

Sodium oleate is a surfactant derived from oleic acid, it has a polar carboxylic head, which is able to adsorb on the surface of the particle, and a long apolar chain of 18 carbon atoms, granting it a significant steric hindrance. It was selected to test the effect of steric hindrance on the colloidal stability of synthesised USPION. Samples MNP_6 and MNP_7 were synthesised with 3% and 1% sodium oleate respectively. Aside from the operative difficulties encountered during the dissolution of the surfactant, the resulting USPION showed very low colloidal stability, settling on the bottom of the reactor after just few minutes from the synthesis. These disappointing results lead to the abandonment of sodium oleate as a stabilizer.

8.2.3.2 APTES

APTES is commonly used in the field of nanomedicine and biomaterials design as a surface charge modifier or as a linker. Its silane head is capable of grafting stably to many oxidic surfaces, forming chemically strong Si—O—M bonds, and its amino functional group serves as a most useful linking tool, as many biologically relevant molecules can be attached to it exploiting the amide or imine chemistry[22,23]. Due to its wide use, it was chosen as a functionalizing molecule to investigate its potential application as both a stabilizing agent and linker. In contrast to the other two compounds, APTES was added on the surface of USPION after their synthesis due to operative issues: while USPION are synthesised in a water-based medium, APTES is susceptible to water hydrolysis and self-polymerization, leading to the loss of control over functionalization. For these reasons, surface modification presented some technical difficulties. A certain amount of USPION prepared with TMAOH (USPt) was functionalized with APTES, producing sample USPION-APTES. Figure 8.5 compares the FTIR spectra of the as-synthesized and APTES-modified samples: the presence of APTES can be clearly observed in the case of USPION-APTES due to the typical vibrational peaks of the organosilane, such as the C—H stretching at 2913 cm^{-1} and 2850 cm^{-1} , the N—H bending at 1558 cm^{-1} , the C—N stretching at 1317 cm^{-1} and the

broad peak of Si—O—Fe stretching at 993 cm^{-1} . Although the functionalization procedure was successful, it had a negative effect on the colloidal stability of treated USPION, as after the addition of APTES the particles aggregated in large clusters and started flocculating, even after being treated with ultrasound. The reason behind this behaviour could lie in the abovementioned self-condensation issues of organosilanes: even if the water amount was limited as much as possible by greatly diluting the USPION suspension in 2-propanol, during functionalization APTES molecules may have reacted with H_2O molecules and polymerized, forming bridges across iron oxide particles and causing their aggregation.

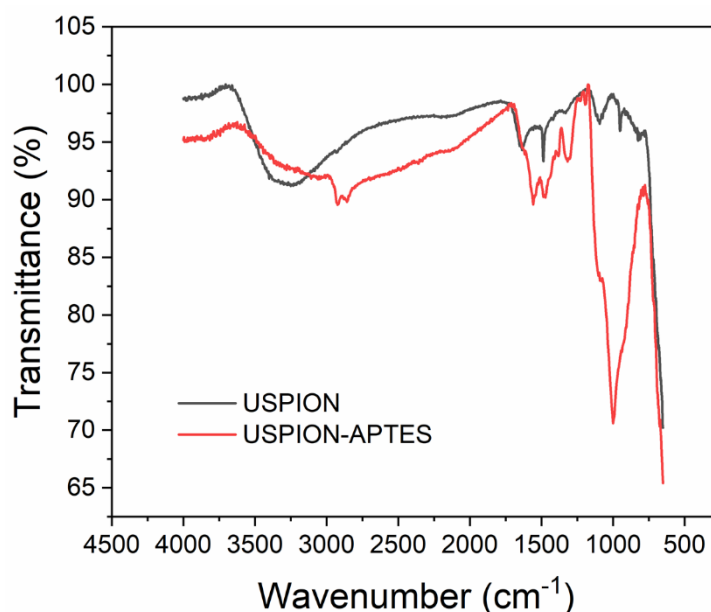


Figure 8.5 - FTIR spectra of bare and APTES-functionalized USPION.

8.2.3.3 Citric acid

Citric acid (together with sodium citrate) is one of the most commonly used stabilizing compounds in the field of nanomedicine and more generally in the field of nanomaterials as a whole[24–27]. Being one of the crucial molecules in many biochemical cycles, its biocompatibility is the highest among the tested compounds. Moreover, citric acid possesses three carboxylic moieties, two thereof are deprotonated at physiological pH of 7.4, thus granting each molecule a double negative charge. For this reason, citric acid acts as a quite effective stabilizer, enhancing same-charge repulsion between nanoparticles and preventing aggregation[28,29].

The first tests involved the addition of citric acid after 20 minutes from the start of the nucleation phase (USPt-CApost), in order to cap already formed nanoparticles with a layer of citrate ions and increase their surface charge density to hinder aggregation. In a second series of experiments, citric acid was added to the precursor solution, before the start of the nucleation phase (USPt-CApre and USPt-CApre05): the rationale behind this choice was to promote the formation of iron-citrate complexes and stop particle growth earlier. Figure 8.6A reports the hydrodynamic diameter of samples USPt, USPt-CApost and USPt-CApre, which respectively represent bare USPION, particles synthesised with citric acid after nucleation and before nucleation. All samples were

later dialysed against water (Figure 8.6B). As can be observed, there is a noticeable effect: before the purification procedure, all three samples have roughly the same colloidal stability, exhibiting size distribution centered around 10 nm. However, after being dialysed against water, the colloidal stability of samples USPt and USPt-CApre visibly suffers from the purification procedure, a phenomenon better explained in Section 8.2.5.3, while sample USPt-CApost seems to be mostly unaffected. These behaviours could possibly be explained on the grounds of the differences in adsorption for citrate added before and after particle precipitation. The addition of citric acid before particle formation may have lead to the incorporation of citrate ions within the iron oxide matrix, while all citrate added after particle growth would have adsorbed on their surface, enhancing its peptizing effect.

However, one of the most interesting distinctions between these samples concerns their stability over time and the difference is appalling: as shown in Figure 8.7, after 5 months, the colloidal stability of sample USPt-CApost_dW appears unaffected, while particles from sample USPt-CApre, even if they were not dialysed, aggregated significantly. This further reinforces the idea that citrate added after particle formation is firmly and densely adsorbed on the surface of iron oxide particles.

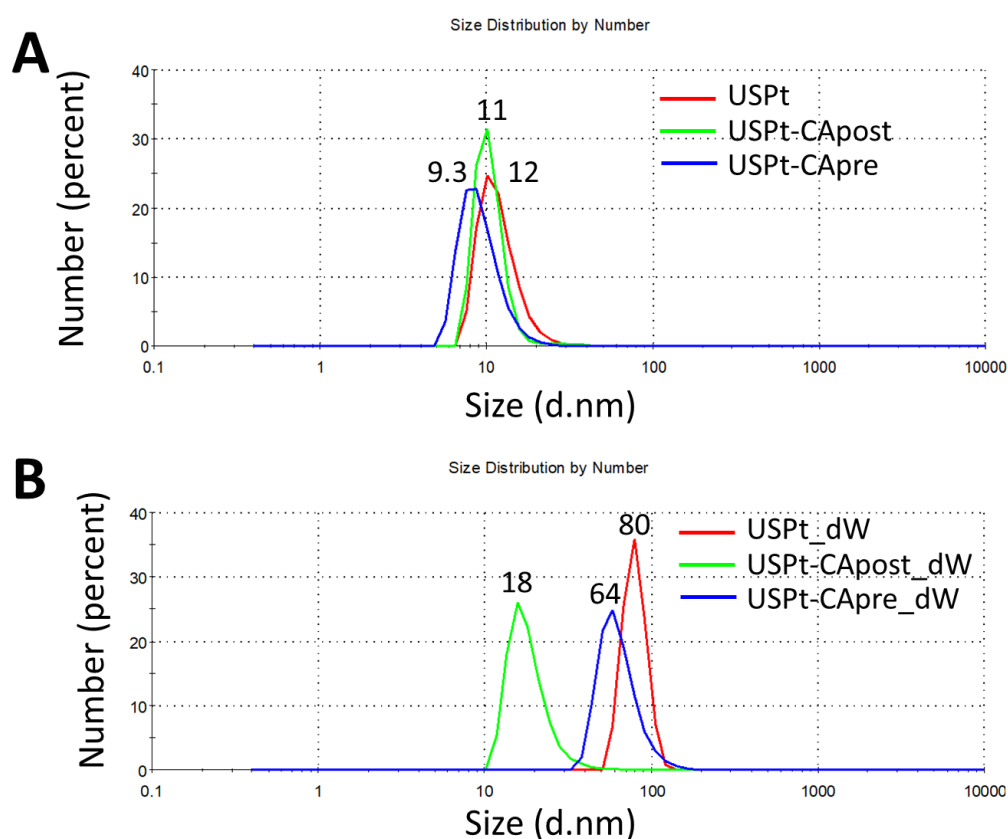


Figure 8.6 - Comparison of the hydrodynamic diameter of USPt, USPt-CApost and USPtCApre before (A) and after dialysis (B).

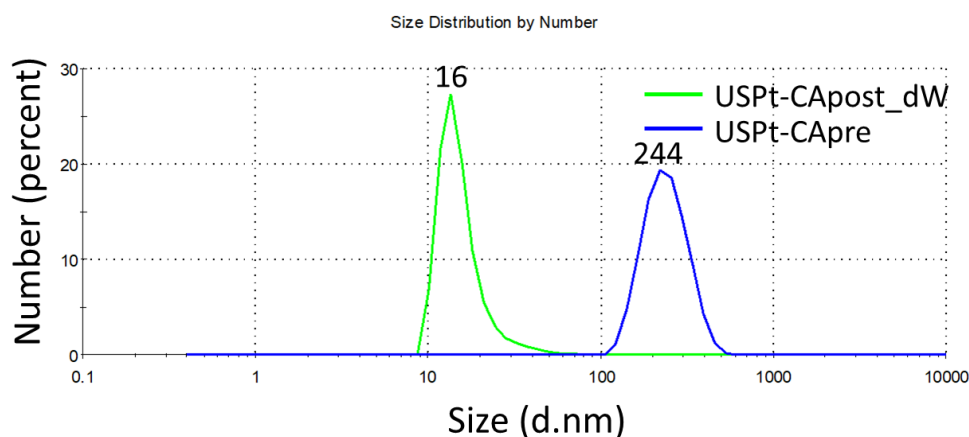


Figure 8.7 - Hydrodynamic diameter of USPt-CApost_dW and USPt-CApre after 5 months.

8.2.4 Effect of precursor concentration

The influence of precursor concentration on the morphology of USPION was investigated by comparing the particles synthesised from 1 M Fe salts solutions with those prepared with 0.5 M precursor solutions.

Figure 8.8 shows TEM images and particle size distributions of sample USPt-CApre, prepared from 1 M solutions, and sample USPt-CApre05, prepared with 0.5 M solutions: the effect on particle size is drastic, as the median value goes from 6.1 ± 0.9 nm to 3.1 ± 0.6 nm. Interestingly, while the particles in sample USPt-CApre seem to be all around the same size, in the case of sample USPt-CApre05 two distinct size distributions are observable: the vast majority of crystallites ranges from 2 to 4 nm but there are also some sparing outliers, which are noticeably larger, around 10 nm. This peculiar size polydispersity may be caused by the lower concentration of the precursors: as there are less free monomers in solution, the growth phase stops earlier, producing overall smaller particles; at the same time, the lower supersaturation degree may create concentration gradients, leading to a heterogeneous nucleation phase, where some nuclei form in advance and start growing at a separate rate from the majority, thus explaining the second larger particle population. Alternatively, the sample moderate polydispersion could be explained through coalescence phenomena[30]. In future experiments, these outliers could be removed by high-speed centrifugation, leaving just the highly monodispersed smaller population.

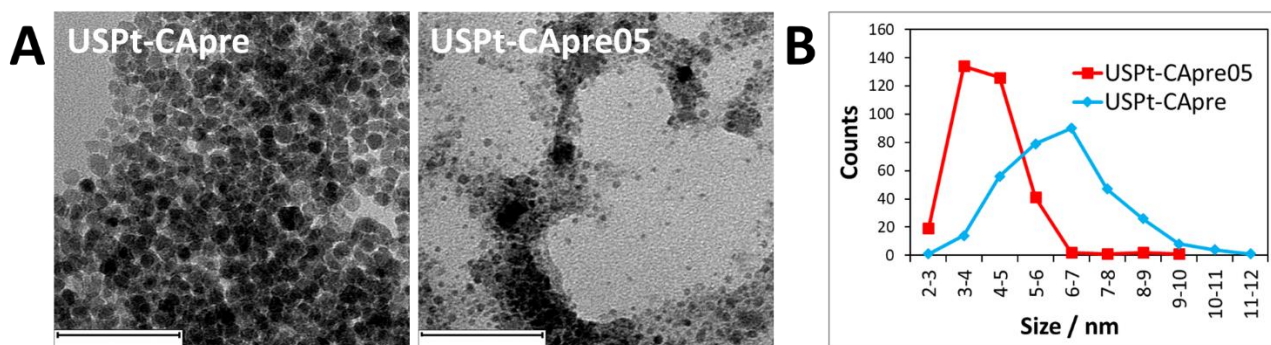


Figure 8.8 - TEM images (A) and size distributions (B) of USPt-CApre and USPt-CApre05. Scale bar is 50 nm.

These results are particularly promising as they represent one of the few reported examples for the preparation of USPIION with a size distribution lower than 4 nm by co-precipitation[31]. The polydispersity issue could possibly be solved by exercising more control over the stirring and base addition rates, to guarantee more homogeneous nucleation and growth conditions. Alternatively, centrifugation cycles could be performed after the synthesis to settle the largest fraction.

8.2.5 Effect of purification procedure

Once prepared, USPIION necessitate of being purified from the remaining reagents, especially the alkaline molecules as they present the greatest cytotoxicity among all components. In this thesis, three different purification methods were explored.

8.2.5.1 Magnetic decantation

This technique is largely utilized when preparing SPION due to its simplicity: a Nd magnet is put underneath the reactor after the synthesis to attract the magnetic nanoparticles to the bottom of the container, allowing for easy washing of the iron oxide. While its ease of use makes it a popular choice, its efficacy is greatly dependant on the magnetic properties and the colloidal stability of the suspension, which can sometimes render magnetic decantation unfeasible as a purification method.

While decantation was effective in purifying some of the least stable samples, such as those prepared with NH_4OH , it proved ineffective when applied to samples with higher colloidal stability, such as those synthesised with TMAOH or even NaOH. Although every sample showed magnetic behaviour and responded to the presence of the Nd magnet, the strength of its magnetic field was not sufficient to settle but the largest particles aggregates. Given these results, it was decided to abandon magnetic decantation as purification technique.

8.2.5.2 Centrifugation

This is the method of choice in the case of non-magnetic nanoparticles. The suspension is subjected to centrifugal force, expressed as Relative Centrifugal Force (RCF), which depends on the particle relative density and size, in order to settle the solid phase on the

bottom of the vial. The supernatant solution containing the impurities is then substituted with clean solvent and the process is repeated for an arbitrary number of cycles. Depending on particle size and colloidal stability, a significantly high value of RCF might be needed to achieve sedimentation.

To force the sedimentation of smaller and more stable nanoparticles, a series of centrifugation protocols, employing different in centrifugal force and timing, were tested on TMAOH-prepared particles. The first test was performed on sample USPt, which was centrifuged at 18000 RCF for 1 h. At the end of the cycle, only a fraction of nanoparticles settled on the bottom of the vial, while most of the population was still suspended in the supernatant. It was decided to greatly increase the RCF to speed up centrifugation/washing cycles. Sample USPt-CAPre was centrifuged at over 360000 RCF for 20 minutes. Even when subjected to such forces, most of the particles were still in suspension at the end of the procedure, but as shown in Figure 8.9B, their size distribution shifted to slightly lower values, going from a median of 6.9 ± 1.1 nm to 6.1 ± 0.9 nm. It was decided to increase centrifugation times drastically, to allow all USPION to sedimentate. The supernatant suspension was centrifuged again at 360000 RCF for 20 h. Unfortunately, the sample vial was compromised during the procedure due to the extreme forces it was exposed to and the sample could not be retrieved. These results suggest that, using the correct combination of RCF and time, centrifugation could prove useful both for purification and size selection of USPION.

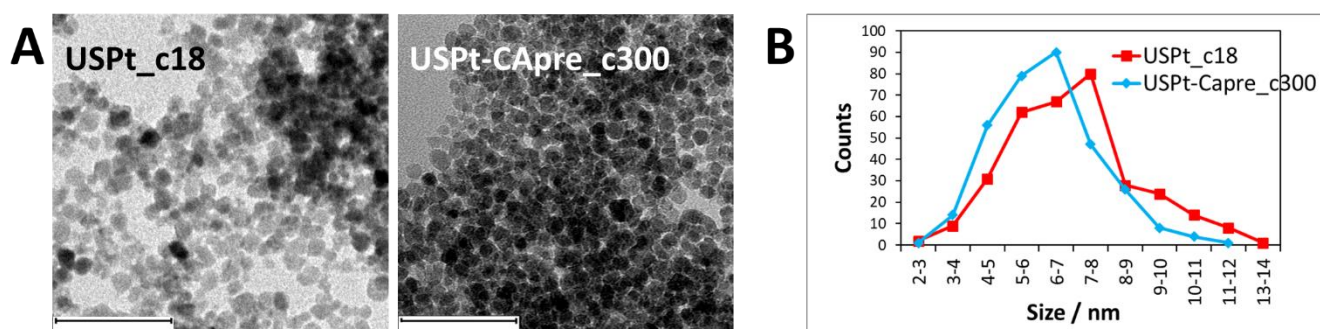


Figure 8.9 - TEM images (A) and particle size distributions (B) of sample USPt_c18 and USPt-CAPre_c300.

8.2.5.3 Dialysis

This technique exploits osmotic pressure to drag impurities out of the supernatant through a semi-permeable membrane. It is generally utilized in the case of fragile particles, which may suffer from the forces involved with centrifugation, or very small and stable nanoparticles, refractory to sedimentation. It is a slow process, compared to the previous two, but it is nonetheless effective whereas other purification techniques fail. As the optimization of a centrifugation procedure proved more difficult than expected, dialysis was tested as a potential way of removing TMAOH excess through osmosis, without the need of settling USPION.

Various dialysis protocols were explored and initially they involved consecutive changes of the medium and longer treatment times, but their effect on colloidal stability

revealed severe. It was thus decided to mitigate this effect by reducing the intensity of the procedure, shortening times and avoiding medium changes, as described in the experimental section. The first experiments gave promising results: after dialysing the sample against Milli-Q water for 24 h, a significant drop of the suspension pH was observed, from 14 to 9, suggesting that most of free TMAOH was being removed by the procedure. Moreover, Figure 8.10 reports the ATR-FTIR spectra of sample USPt-CApre05 before and after dialysis: it can be noticed that the vibrational peaks relative to TMAOH, notably the asymmetric and symmetric C—H bending between 1488 cm^{-1} and 1342 cm^{-1} and the C—N stretching at 950 cm^{-1} , appear much less intense after the procedure. Although the successful removal of a part of TMAOH represented a positive outcome, the procedure had a moderate negative effect on the colloidal stability of the sample. As shown in Figure 8.11A, the hydrodynamic diameter of suspended particles shifted from an optimal value of 9.3 nm to 15 nm: this result could be explained by the decrease in TMAOH concentration and subsequent removal of a stabilizing $\text{N}(\text{CH}_3)_4^+$ layer on the particles, leading to increased aggregation.

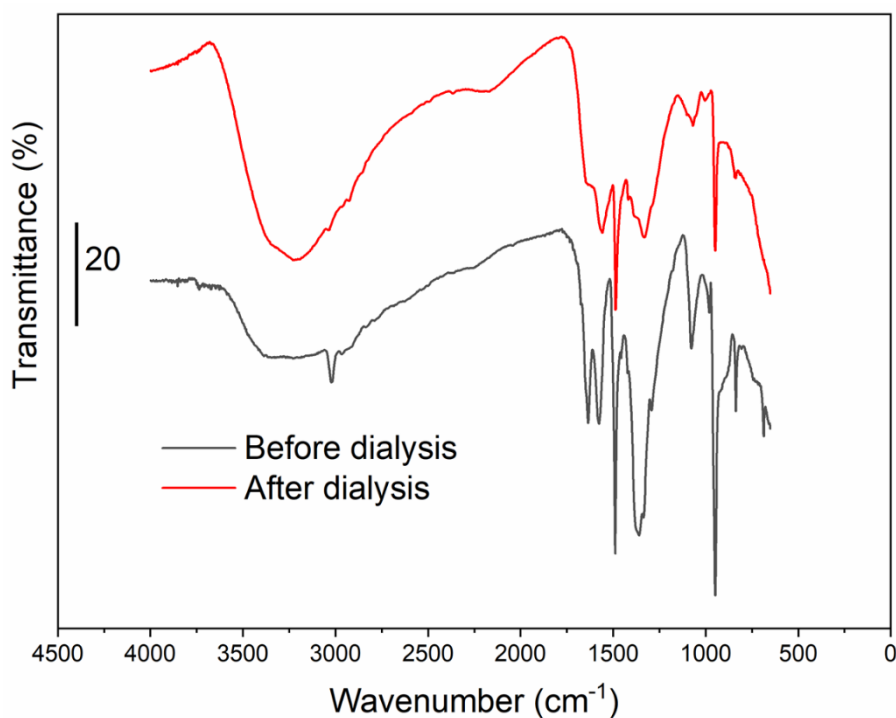


Figure 8.10 - Comparison between the ATR-FTIR spectra of sample USPt-CApre05 before and after dialysis was performed.

A second test was performed on sample USPt-CApre. This batch of USPION was synthesised using citric acid in conjunction with TMAOH, with the aim of substituting the toxic hydroxide with a biocompatible stabilizing agent. For this reason, sample USPt-CApre was dialysed against a 0.08 M solution of citric acid, to see if the purification procedure would be able to substitute the peptizing agents without lowering colloidal stability. This time, the effect on the hydrodynamic diameter was slightly more impactful, as shown in Figure 8.11B, with a slight shift from 9.3 nm to 24 nm.

Unfortunately, after few days from the procedure, the suspension had changed colour, from black to brown, and the particles showed no magnetic behaviour. This phenomenon is probably linked to the acidic environment the USPION were left into after the dialysis, which after dialysis reached pH 2.5: it was reported that acidic conditions may favour the oxidation of magnetite to maghemite and eventually to free Fe^{3+} ions, following the mechanism reported in Scheme 8.1[32,33]. To avoid the degradation of USPION, a third test was performed on sample USPt-CApre05. This time, USPION were dialysed against a 0.08 M sodium citrate solution, to exploit the peptizing effect of the citrate ions without lowering the suspension pH. As expected, no degradation was observed in this case, the suspension retained its original black colour and responded to magnetic fields. Interestingly, particle aggregation was the most pronounced among all dialysis solutions, as shown by the DLS charts in Figure 8.11C: the hydrodynamic diameter went from 9.9 nm to 141 nm, with a short, broad secondary peak around 1100 nm.

As dialysis can be very time consuming, a new approach based on the use of dialysing centrifuge vials was tested. Sample USPt-CApre05_dW was subjected to 4 cycles of centrifuge-aided dialysis against pure water, while the amount of TMAOH was monitored through FTIR spectroscopy. As can be seen in Figure 8.12, the signals relative to TMAOH lose intensity with each dialysis cycle and have almost disappeared by the fourth. As each of said cycles takes about 10 minutes, this procedure represents a viable fast purification method to quickly wash small amount of sample.

Based on these results, it can be concluded that while dialysis is a very promising purification technique, the specific conditions to adopt need to be finely tuned. Pure water appears to provide the best outcome, avoiding particle dissolution and keeping the suspension sufficiently stable. As previously reported in Section 8.2.3.3, colloidal stability further improves if citric acid is added after particle formation, at which point the suspension can maintain stable for months.

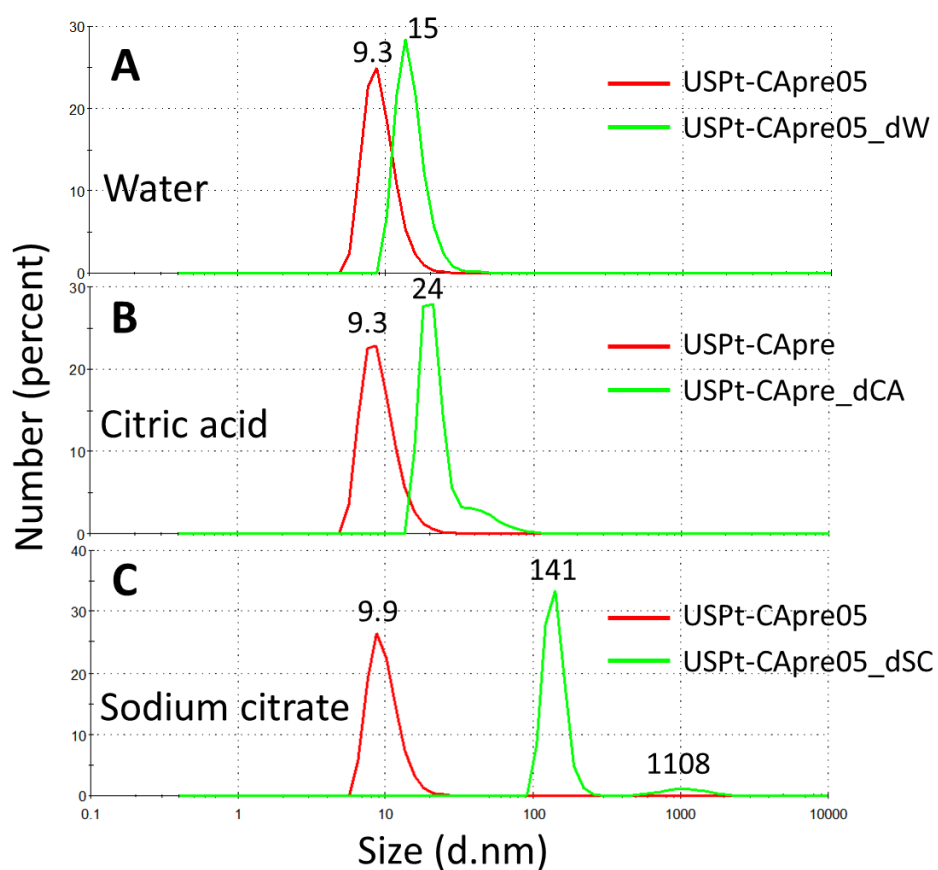
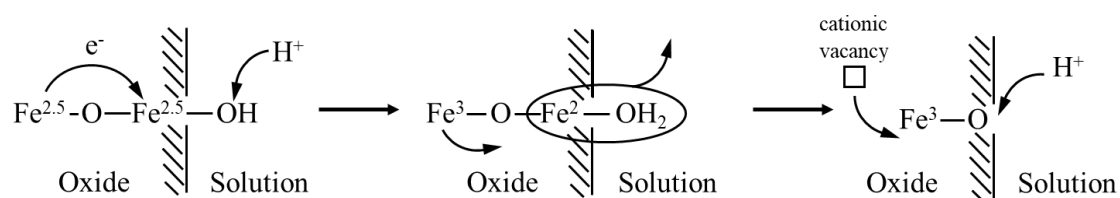


Figure 8.11 - Comparison between the effect of dialysis against different media on the colloidal stability of treated samples: USPt-CApre05 (A, C) and USPt-CApre (B).



Scheme 8.1 - Mechanism of magnetite dissolution promoted by acidic conditions. Figure adapted from [32].

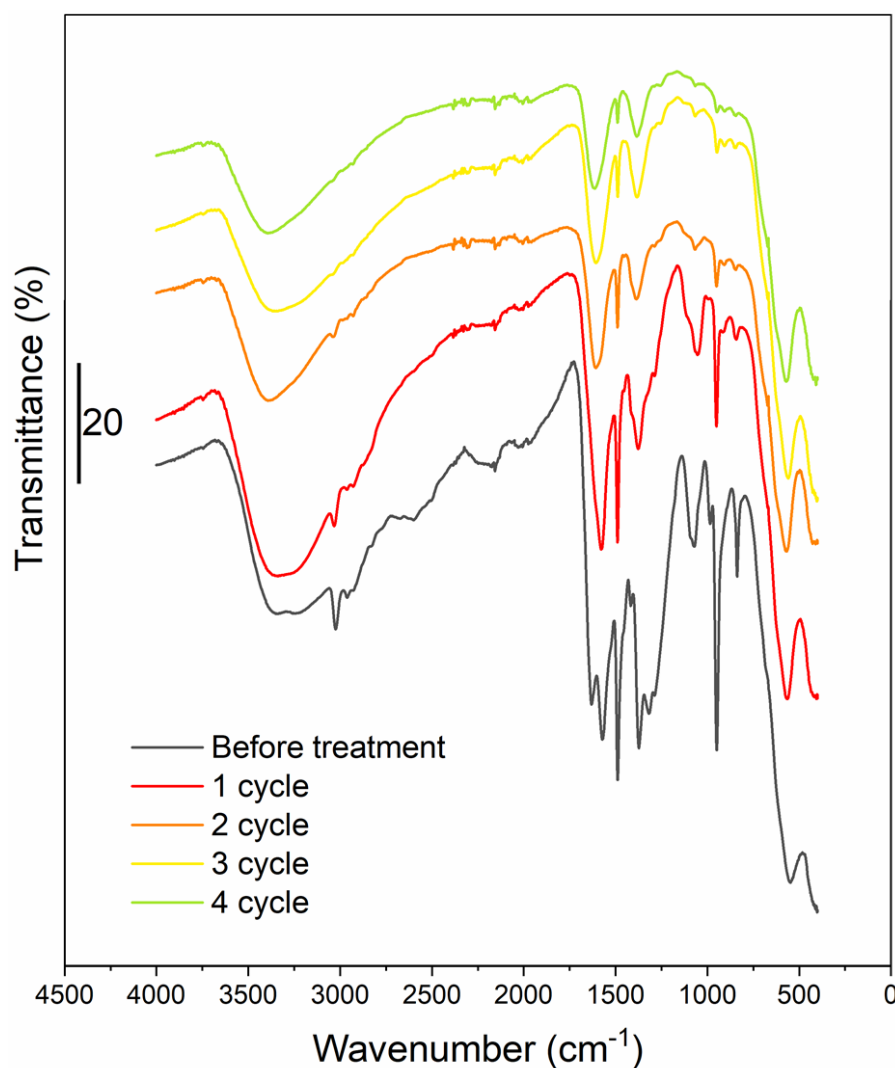


Figure 8.12 - Comparison between the ATR-FTIR spectra of sample USPt-CApre05_dW before and after several cycles of centrifuge-assisted dialysis.

8.2.6 Isoelectric point

The isoelectric point of selected samples was evaluated through ζ -potential measurements at different pH values. One of the most interesting findings is that citrate surface adsorption can be closely followed by looking at the pH_{IEP} values of samples USPt, USPt-CApre05, USPt-CApre05_dW and USPt-CApost_dW, shown in Figure 8.13. As can be noticed, the pH_{IEP} of USPt measures 5.7, perfectly in line with values reported in the literature for magnetite nanoparticles[34], but with the implementation of citric acid, in sample USPt-CApre05, it drops to 1.96, due to all the negatively charged citrate ions adsorbed on the particles surface. After being dialysed, some of these citrate ions desorb, increasing the surface charge, thus raising the pH_{IEP} of sample USPt-CApre05_dW to 2.85. The effect on the pH_{IEP} is less pronounced for sample USPt-CApost_dW, possibly because, as suggested by the superior stability exhibited by this sample (Figure 8.6B), citrate ions are more homogeneously adsorbed on the surface and form a stronger coating on particles.

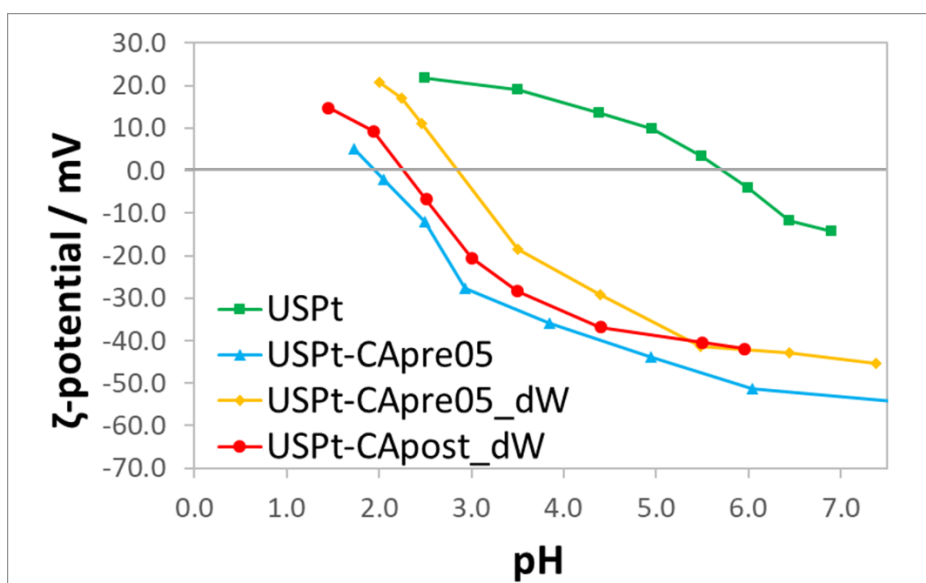


Figure 8.13 - Z-potential curves of citrate-functionalized samples.

8.2.7 Reproducibility

The reproducibility of these synthetic protocols can be evaluated by looking at the comparison between the presented samples and those prepared previously by a different operator: for example, sample USPt, showing a size median of 7.3 ± 1.3 nm, had been previously synthesised and measured at 7.7 ± 1.6 nm. Moreover, samples USPt-CApre and USPt-CApre05 were repeatedly synthesised (Figure 8.14), measuring 6.2 ± 1.1 nm and 6.1 ± 0.9 nm for the first and 3.0 ± 0.7 nm and 3.1 ± 0.6 nm for the second.

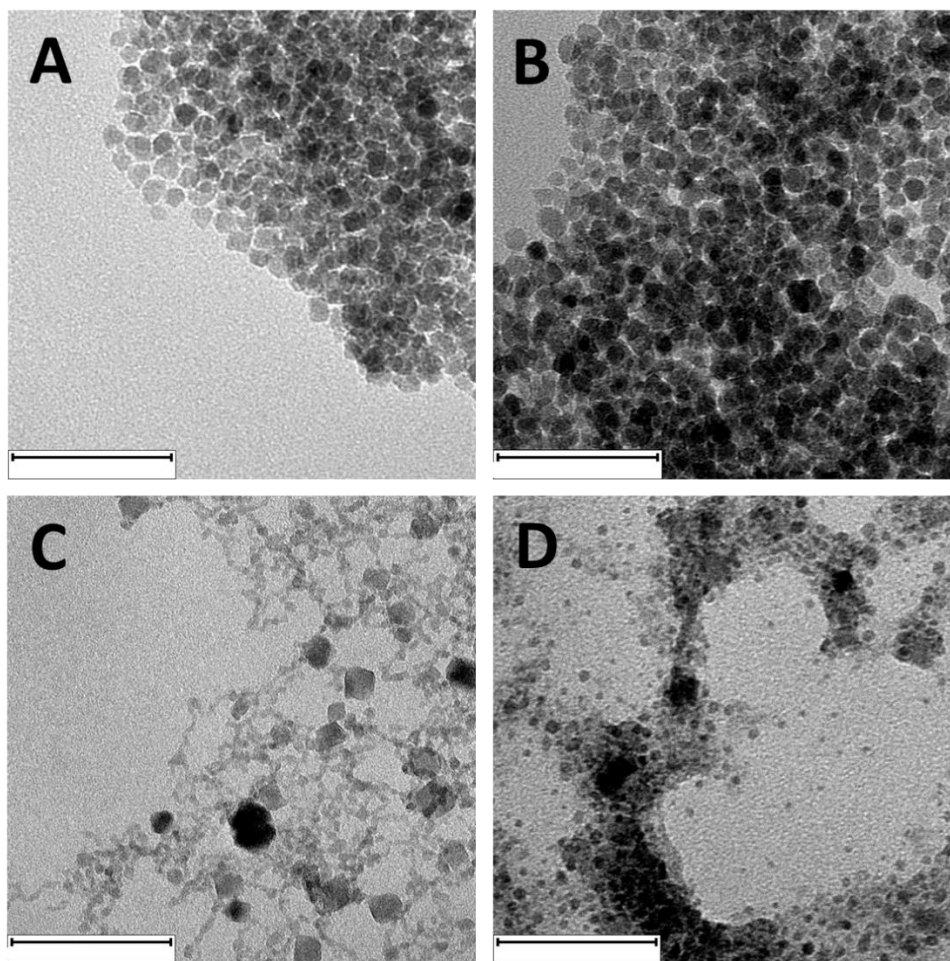


Figure 8.14 - TEM images illustrating the repeated synthesis of USPt-CApre (A, B) and USPt-CApre05 (C, D). Scale bar is 50 nm.

8.3 Conclusions

The co-precipitation synthesis of Ultrasmall Iron Oxide Superparamagnetic Nanoparticles was studied and optimized, investigating the influence of several parameters such as reaction temperature, base type, purification procedure, peptizing agent and precursor concentration. It was found that the best results were obtained at room temperature and that the peptizing effect of the tetramethylammonium ion is crucial to guarantee an optimal colloidal stability, making TMAOH the base of choice. The concentration of starting precursor solutions proved to be the determining factor acting on particle size, as halving it led to a narrow particle size distribution centred around 3 nm, a significant shift from the starting 7 nm. Centrifugation was ineffective when adopted to settle and wash the nanoparticles, but it proved a promising size-selection tool. Dialysis against pure water proved to be the most efficient technique to remove potentially toxic impurities, especially when combined with centrifugation to achieve an efficient work up protocol, although it caused slight repercussions on colloidal stability, which were mitigated by the use of a proper stabilizing agent. To preserve a high colloidal stability even after the removal of TMAOH, surface

modification with several stabilizing agents was tested. Among the tested molecules, citric acid was the only one to show positive effects on particle size and aggregation, more so when added after the formation of USPIO, in which case the suspension proved stable even after 5 months of ambient conditions storage. These results represent a promising advance in the development of efficient T1 contrast agents based on USPIO in terms of lowering the synthetic requirements: monodisperse, stable magnetic nanoparticles were in fact prepared through a simple co-precipitation procedure, performed at room temperature, without the aid of any polymeric additive. Future work will concern the phase identification of purified USPIO via X-ray diffraction and X-ray photoelectron spectroscopy, which could not be performed due to time constraints. Furthermore, relaxivity measurements will be carried out on the most promising samples. The next step will then be exploring the surface functionalization of these USPIO with functional molecule devoted to selective targeting, possibly antibodies fragments.

8.4 References

- [1] Y. Okuhata, Delivery of diagnostic agents for magnetic resonance imaging, *Adv. Drug Deliv. Rev.* 37 (1999) 121–137. doi:10.1016/S0169-409X(98)00103-3.
- [2] M. Rogosnitzky, S. Branch, Gadolinium-based contrast agent toxicity: a review of known and proposed mechanisms, *BioMetals*. 29 (2016) 365–376. doi:10.1007/s10534-016-9931-7.
- [3] T. Kanda, K. Ishii, H. Kawaguchi, K. Kitajima, D. Takenaka, High Signal Intensity in the Dentate Nucleus and Globus Pallidus on Unenhanced T1-weighted MR Images: Relationship with Increasing Cumulative Dose of a Gadolinium-based Contrast Material, *Radiology*. 270 (2014) 834–841. doi:10.1148/radiol.13131669.
- [4] J. Ramalho, R.C. Semelka, M. Ramalho, R.H. Nunes, M. AlObaidy, M. Castillo, Gadolinium-Based Contrast Agent Accumulation and Toxicity: An Update, *Am. J. Neuroradiol.* 37 (2016) 1192–1198. doi:10.3174/ajnr.A4615.
- [5] N. Lee, T. Hyeon, Designed synthesis of uniformly sized iron oxide nanoparticles for efficient magnetic resonance imaging contrast agents, *Chem. Soc. Rev.* 41 (2012) 2575–2589. doi:10.1039/C1CS15248C.
- [6] Z. Li, P.W. Yi, Q. Sun, H. Lei, H. Li Zhao, Z.H. Zhu, S.C. Smith, M.B. Lan, G.Q.M. Lu, Ultrasmall Water-Soluble and Biocompatible Magnetic Iron Oxide Nanoparticles as Positive and Negative Dual Contrast Agents, *Adv. Funct. Mater.* 22 (2012) 2387–2393. doi:10.1002/adfm.201103123.
- [7] J.-C. Brisset, M. Sigovan, F. Chauveau, A. Riou, E. Devillard, V. Desestret, M. Touret, S. Nataf, J. Honnorat, E. Canet-Soulas, N. Nighoghossian, Y. Berthezene, M. Wiart, Quantification of Iron-Labeled Cells with Positive Contrast in Mouse Brains, *Mol. Imaging Biol.* 13 (2011) 672–678. doi:10.1007/s11307-010-0402-1.
- [8] C. Lin, S. Cai, J. Feng, Positive Contrast Imaging of SPIO Nanoparticles, *J.*

Nanomater. 2012 (2012) 1–9. doi:10.1155/2012/734842.

- [9] H. Wei, O.T. Bruns, M.G. Kaul, E.C. Hansen, M. Barch, A. Wiśniowska, O. Chen, Y. Chen, N. Li, S. Okada, J.M. Cordero, M. Heine, C.T. Farrar, D.M. Montana, G. Adam, H. Ittrich, A. Jasanoff, P. Nielsen, M.G. Bawendi, Exceedingly small iron oxide nanoparticles as positive MRI contrast agents, *Proc. Natl. Acad. Sci.* 114 (2017) 2325–2330. doi:10.1073/pnas.1620145114.
- [10] Y. Bao, J.A. Sherwood, Z. Sun, Magnetic iron oxide nanoparticles as T1 contrast agents for magnetic resonance imaging, *J. Mater. Chem. C* 6 (2018) 1280–1290. doi:10.1039/C7TC05854C.
- [11] R. Hachani, M. Lowdell, M. Birchall, A. Hervault, D. Mertz, S. Begin-Colin, N.T.K. Thanh, Polyol synthesis, functionalisation, and biocompatibility studies of superparamagnetic iron oxide nanoparticles as potential MRI contrast agents, *Nanoscale* 8 (2016) 3278–3287. doi:10.1039/C5NR03867G.
- [12] R. Bhavesh, A. Lechuga-Vieco, J. Ruiz-Cabello, F. Herranz, T1-MRI Fluorescent Iron Oxide Nanoparticles by Microwave Assisted Synthesis, *Nanomaterials* 5 (2015) 1880–1890. doi:10.3390/nano5041880.
- [13] B.H. Kim, N. Lee, H. Kim, K. An, Y. Il Park, Y. Choi, K. Shin, Y. Lee, S.G. Kwon, H. Bin Na, J.-G. Park, T.-Y. Ahn, Y.-W. Kim, W.K. Moon, S.H. Choi, T. Hyeon, Large-Scale Synthesis of Uniform and Extremely Small-Sized Iron Oxide Nanoparticles for High-Resolution T1 Magnetic Resonance Imaging Contrast Agents, *J. Am. Chem. Soc.* 133 (2011) 12624–12631. doi:10.1021/ja203340u.
- [14] M. Hatakeyama, H. Kishi, Y. Kita, K. Imai, K. Nishio, S. Karasawa, Y. Masaike, S. Sakamoto, A. Sandhu, A. Tanimoto, T. Gomi, E. Kohda, M. Abe, H. Handa, A two-step ligand exchange reaction generates highly water-dispersed magnetic nanoparticles for biomedical applications, *J. Mater. Chem.* 21 (2011) 5959. doi:10.1039/c0jm04381h.
- [15] M. Fang, V. Ström, R.T. Olsson, L. Belova, K. V Rao, Particle size and magnetic properties dependence on growth temperature for rapid mixed co-precipitated magnetite nanoparticles, *Nanotechnology* 23 (2012) 145601. doi:10.1088/0957-4484/23/14/145601.
- [16] J. Sun, S. Zhou, P. Hou, Y. Yang, J. Weng, X. Li, M. Li, Synthesis and characterization of biocompatible Fe₃O₄ nanoparticles, *J. Biomed. Mater. Res. Part A* 80A (2007) 333–341. doi:10.1002/jbm.a.30909.
- [17] Â.L. Andrade, J.D. Fabris, J.D. Ardisson, M.A. Valente, J.M.F. Ferreira, Effect of Tetramethylammonium Hydroxide on Nucleation, Surface Modification and Growth of Magnetic Nanoparticles, *J. Nanomater. 2012* (2012) 1–10. doi:10.1155/2012/454759.
- [18] A. Donev, Improving the Density of Jammed Disordered Packings Using Ellipsoids, *Science* (80-.). 303 (2004) 990–993. doi:10.1126/science.1093010.
- [19] R.P. Araújo-Neto, E.L. Silva-Freitas, J.F. Carvalho, T.R.F. Pontes, K.L. Silva, I.H.M. Damasceno, E.S.T. Egito, A.L. Dantas, M.A. Morales, A.S. Carriço, Monodisperse sodium oleate coated magnetite high susceptibility nanoparticles for hyperthermia applications, *J. Magn. Magn. Mater.* 364 (2014) 72–79. doi:10.1016/j.jmmm.2014.04.001.
- [20] P. Padwal, R. Bandyopadhyaya, S. Mehra, Biocompatible citric acid-coated iron oxide nanoparticles to enhance the activity of first-line anti-TB drugs in *Mycobacterium*

smegmatis, J. Chem. Technol. Biotechnol. 90 (2015) 1773–1781. doi:10.1002/jctb.4766.

- [21] A. Javid, S. Ahmadian, A.A. Saboury, S.M. Kalantar, S. Rezaei-Zarchi, S. Shahzad, Biocompatible APTES–PEG Modified Magnetite Nanoparticles: Effective Carriers of Antineoplastic Agents to Ovarian Cancer, Appl. Biochem. Biotechnol. 173 (2014) 36–54. doi:10.1007/s12010-014-0740-6.
- [22] T. Taroni, S. Cauteruccio, R. Vago, S. Franchi, N. Barbero, E. Licandro, S. Ardizzzone, D. Meroni, Thiahelicene-grafted halloysite nanotubes: Characterization, biological studies and pH triggered release, Appl. Surf. Sci. 520 (2020) 146351. doi:10.1016/j.apsusc.2020.146351.
- [23] M. Massaro, R. Amorati, G. Cavallaro, S. Guernelli, G. Lazzara, S. Milioto, R. Noto, P. Poma, S. Riela, Direct chemical grafted curcumin on halloysite nanotubes as dual-responsive prodrug for pharmacological applications, Colloids Surfaces B Biointerfaces. 140 (2016) 505–513. doi:10.1016/j.colsurfb.2016.01.025.
- [24] J.-W. Park, J.S. Shumaker-Parry, Structural Study of Citrate Layers on Gold Nanoparticles: Role of Intermolecular Interactions in Stabilizing Nanoparticles, J. Am. Chem. Soc. 136 (2014) 1907–1921. doi:10.1021/ja4097384.
- [25] W.C. Poller, N. Löwa, M. Schleicher, A. Münster-Wandowski, M. Taupitz, V. Stangl, A. Ludwig, F. Wiekhorst, Initial interaction of citrate-coated iron oxide nanoparticles with the glycocalyx of THP-1 monocytes assessed by real-time magnetic particle spectroscopy and electron microscopy, Sci. Rep. 10 (2020) 3591. doi:10.1038/s41598-020-60162-8.
- [26] A.P.F. Monteiro, L.D. Caminhas, J.D. Ardisson, R. Paniago, M.E. Cortés, R.D. Sinisterra, Magnetic nanoparticles coated with cyclodextrins and citrate for irinotecan delivery, Carbohydr. Polym. 163 (2017) 1–9. doi:10.1016/j.carbpol.2016.11.091.
- [27] Y. Jabalera, F. Oltolina, M. Prat, C. Jimenez-Lopez, J.F. Fernández-Sánchez, D. Choquesillo-Lazarte, J. Gómez-Morales, Eu-Doped Citrate-Coated Carbonated Apatite Luminescent Nanoprobes for Drug Delivery, Nanomaterials. 10 (2020) 199. doi:10.3390/nano10020199.
- [28] H. Al-Johani, E. Abou-Hamad, A. Jedidi, C.M. Widdifield, J. Viger-Gravel, S.S. Sangaru, D. Gajan, D.H. Anjum, S. Ould-Chikh, M.N. Hedhili, A. Gurinov, M.J. Kelly, M. El Eter, L. Cavallo, L. Emsley, J.-M. Basset, The structure and binding mode of citrate in the stabilization of gold nanoparticles, Nat. Chem. 9 (2017) 890–895. doi:10.1038/nchem.2752.
- [29] M.A. Dheyab, A.A. Aziz, M.S. Jameel, O.A. Noqta, P.M. Khaniabadi, B. Mehrdel, Simple rapid stabilization method through citric acid modification for magnetite nanoparticles, Sci. Rep. 10 (2020) 10793. doi:10.1038/s41598-020-67869-8.
- [30] N.T.K. Thanh, N. Maclean, S. Mahiddine, Mechanisms of Nucleation and Growth of Nanoparticles in Solution, Chem. Rev. 114 (2014) 7610–7630. doi:10.1021/cr400544s.
- [31] G. Wang, X. Zhang, A. Skallberg, Y. Liu, Z. Hu, X. Mei, K. Uvdal, One-step synthesis of water-dispersible ultra-small Fe₃O₄ nanoparticles as contrast agents for T1 and T2 magnetic resonance imaging, Nanoscale. 6 (2014) 2953. doi:10.1039/c3nr05550g.
- [32] J.-P. Jolivet, C. Chanéac, E. Tronc, Iron oxide chemistry. From molecular clusters to extended solid networks, Chem. Commun. (2004) 477–483. doi:10.1039/B304532N.

- [33] S. Briceño, A.C. Hernandez, J. Sojo, L. Lascano, G. Gonzalez, Degradation of magnetite nanoparticles in biomimetic media, *J. Nanoparticle Res.* 19 (2017) 140. doi:10.1007/s11051-017-3800-3.
- [34] J.L. Viota, F.J. Arroyo, A.V. Delgado, J. Horno, Electrokinetic characterization of magnetite nanoparticles functionalized with amino acids, *J. Colloid Interface Sci.* 344 (2010) 144–149. doi:10.1016/j.jcis.2009.11.061.

Conclusions and outlooks

In my Ph.D. work I wanted to highlight the central role of nanostructured materials for the development of cutting-edge technological devices.

To this purpose, my research was devoted to the study of widely different oxides, dedicating a substantial amount of time to the thorough characterization of their morphology and surface properties, before focusing on their modification with several functional molecules, each apt to confer the base material specific abilities to perform a wide array of tasks. Embracing the versatile nature of oxides, this thesis covered diverse topics, all linked by the leitmotiv of applicative oxide modification: the unique morphology and surface properties of halloysite nanotubes, along with their selective functionalization by stimuli-responsive covalent systems, were carefully investigated and employed to design pH-responsive drug delivery devices and resistant polymer nanocomposites. The first proved to efficiently discriminate between critical physiological pH values, suggesting their potential as targeting drug carriers, while the latter greatly improved the hydrothermal resistance of pristine polyamide 6, possibly expanding the range of its current applications. The wetting, morphological and photocatalytical properties of TiO₂ films were vastly varied and finely tuned to produce functional coatings that could prove useful for most diverse applications, from self-cleaning superhydrophobic surfaces, to anti-fouling, photo-regenerative sensor coatings, up to antimicrobial active layers for food packaging. More specifically, photoactive porous coatings not only retained the bare electrode electrochemical properties, but they even enhanced them, all while efficiently degrading organic contaminations upon UV light irradiation. Moreover, films functionalized with cinnamaldehyde were showed to actively release it under mild acidic conditions. Finally, the facile co-precipitation synthesis of ultras-small superparamagnetic iron oxide nanoparticles was optimized through the scrupulous evaluation of numerous synthetic parameters, as well as surface modifications. It was found that the most influential parameter determining particles size is precursor concentration, the tuning thereof allowed to obtain monodispersed particles of around 3 nm. The peptizing role of tetramethylammonium and citrate ions was demonstrated to be crucial and was exploited to promote colloidal stability up to 5 months. These results highlight the potential of prepared USPIO as MRI T1 contrast agents.

Some of the queries presented in this thesis represent the first step of promising new studies marked by a wider scope. For example, the studies on halloysite-based polymeric nanocomposites may be furthered by exploring the possibility of using a biopolymer as the matrix, to develop moisture resistant advanced materials for biomedical applications, such as tissue engineering and bone implants. Similarly, the work on TiO₂ porous films could be expanded: EISA films presented in Chapter 6, exhibiting higher surface area but lower photocatalytical properties, could potentially allow greater amounts of cinnamaldehyde to be released at the cost of slower regeneration rates. Moreover, the same synthetic approach adopted to prepare porous TiO₂ films could be replicated on other metal oxide systems to employ their versatile properties in a multitude of additional scenarios. Moreover, the research on USPIO-

based T1 contrast agents is just at the beginning: now that a simple, reproducible synthetic protocol has been established, the next step will concern the detailed study of their crystalline and magnetic properties to complete their physicochemical picture. Once a firm knowledge base on these particles will have been built, then efforts could be made to modify their surface and provide them targeting features, turning them into selective imaging devices.

Publications

- C. Cionti, T. Taroni, V. Sabatini, D. Meroni, **Nanostructured oxide-based systems for the pH-triggered release of cinnamaldehyde**, *Materials*, 2021, 14, 1536, DOI: 10.3390/ma14061536
- V. Sabatini, T. Taroni, R. Rampazzo, M. Bompieri, D. Maggioni, D. Meroni, M. A. Ortenzi, S. Ardizzone, **PA6 and Halloysite Nanotubes Composites with Improved Hydrothermal Ageing Resistance: Role of Filler Physicochemical Properties, Functionalization and Dispersion Technique**, *Polymers*, 2020, 12, 1, 211, DOI: 10.3390/polym12010211
- T. Taroni, S. Cauteruccio, R. Vago, S. Franchi, N. Barbero, E. Licandro, S. Ardizzone, D. Meroni, **Thiahelicene-grafted halloysite nanotubes: Characterization, biological studies and pH triggered release**, *Applied Surface Science*, 2020, 520, 146351, DOI: 10.1016/j.apsusc.2020.146351
- T. Taroni, D. Meroni, L. Rimoldi, K. Fidecka, S. Ardizzone, **Study of Halloysite nanotubes selective functionalization with phosphonic acids, supported by synthetic oxide models**, *Applied Surface Science*, 2019, 486, 466, DOI: 10.1016/j.apsusc.2019.04.264
- C. Cionti, T. Taroni, D. Meroni, **Bouncing droplets: a hands-on activity to demonstrate the properties and applications of superhydrophobic surface coatings**, *Journal of Chemical Education*, 2019, 96, 1971, DOI: 10.1021/acs.jchemed.9b00406
- L. Rimoldi, T. Taroni, D. Meroni, **Wetting modification by photocatalysis: A hands-on activity to demonstrate photoactivated reactions at semiconductor surfaces**, *Journal of Chemical Education*, 2019, 95 (12), 2216, DOI: 10.1021/acs.jchemed.8b00362
- T. Taroni, D. Meroni, L. Rossi, **Simple co-precipitation synthesis of USPION suitable as T1 MRI contrast agents**, in preparation